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Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth

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ABSTRACT

This work showed for the first time that organic nutrient transformation techniques based on locally available materials (manure, green waste and advanced biochar) can increase fertilizing efficiency of the resulting substrate by a factor of three compared with other organic amendments without biochar. We used three different composting methods to investigate the techniques of organic nutrient transformations; i) conventional composting (composting process completed without turning the piles) ii) aerobic composting (composting process with manual turning of piles) and iii) bokashi composting (anaerobic lacto-fermentation). Composting was carried out in the absence (compost alone) and the presence of biochar (co-composted). Biochar was produced locally from an invasive forest shrub '*Eupatorium adenophorum*'. A pot trial with maize grown in silty loam soil was carried out to investigate the agronomic effect produced using three above-mentioned composting methods that were compared with conventional mineral fertilizers (NPK). Significant effects of co-composted bokashi-biochar (60 t ha⁻¹) were observed on maize growth, which increased biomass by 243% compared to mineral NPK, also showing better growth effects than conventional and aerobic composting amendments. Improved soil available nutrients (available P and other exchangeable base cations (K⁺, Ca²⁺ and Mg²⁺)) were probably the cause of the superior growth effect of co-composted bokashi-biochar.

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Introduction

Biochar amendment, either alone or in combined with organic or mineral fertilizers, to low productive tropical soils, has been recognized as an efficient and sustainable method to improve farm productivity (Lehmann and Rondon 2006; Liang et al. 2006; Yamato et al. 2006; Martinsen et al. 2014; Sanger et al. 2017). Biochar addition has resulted in improved soil physicochemical properties such as pH, cation exchange capacity (CEC), base saturation (BS) and water-holding capacity (Glaser et al. 2002; Chan et al. 2008; Cornelissen et al. 2013; Chintala et al. 2014; Obia et al. 2016; Naeem et al. 2017) as well as biological properties such as enhanced microbial activities (Atkinson et al. 2010; Ye et al. 2016).

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Recently, biochar-compost mixtures have been investigated as a method to produce effective biochar-based slow release organic fertilizers (Schmidt 2012; Ye et al. 2016). Biochar can either be mixed with composting materials during the composting process, i.e. 'co-composted', or added directly to stored matured compost (Vandecasteele et al. 2016). Addition of biochar during the composting process changes the compost properties, and quality and can lead to improved physicochemical properties (organic carbon content (OC), pH, moisture content) and nutrient availability (nitrogen, phosphorous and other important nutrients) in the end product (Prost et al. 2013; Zhang and Sun 2014; Agegnehu et al. 2016; Vandecasteele et al. 2016). The co-composting process results in an organic coating on the biochar particles which reduces the hydrophobicity of biochar and improves nutrient retention conditions leading to improved agronomic performance (Kammann et al. 2015; Hagemann et al. 2017; Joseph et al. 2017). This organic coating on the biochar particles also may affect soil redox (Eh) status (Hagemann et al. 2017). Plants are affected by very low and very high Eh or pH, and these parameters should be kept at a medium level for optimal performance (Husson 2013). The application of highly oxidized co-composted biochar (with high Eh) could have a positive agronomic impact on highly reduced soils, however, for oxidized conditions in aerobic soils, high Eh could negatively affect the soil-plant-microorganism system and crop production (Husson 2013). In such cases, strongly reduced low-Eh bokashi fermented biochar (lacto-fermentation) could have a positive effect on highly oxidized soils thereby maintaining a healthy soil ecosystem and better crop yield (Husson 2013).

In a recent field study, the amendment of co-composted biochar to a tropical Ferralsol increased maize crop grain and biomass production by 10–29% and 9–18%, respectively, when compared to inorganic fertilizers application (Agegnehu et al. 2016). Similarly, in a temperate infertile sandy soil, biochar (40 t ha⁻¹) co-composted with various organic materials showed at least the same agronomic performance compared to mineral fertilization under optimum agronomic conditions (Glaser et al. 2015). In pot experiments, co-composted biochar (2% w/w) was observed to quadruple plant growth of *Chenopodium quinoa* in a nutrient-poor sandy soil compared to the non-amended control (Kammann et al. 2015). Another recent field study (Schmidt et al. 2017) conducted in moderately acidic Nepalese silty loam soils demonstrated a significant agronomic benefit of biochar combined with organic fertilizers (cow urine and manure) when compared with organic fertilization alone and NPK-biochar fertilization. However, systematic and mechanistic trials to understand the agronomic effects of various biochar-compost formulations are currently lacking.

In addition, a further area currently left unexplored is the use of biochar in bokashi fermentation (anaerobic lactic fermentation), which uses manure and bio-waste products to produce high-value soil amendments (Dreschke et al. 2015; Probst et al. 2015). Bokashi fermentation (Japanese term for 'fermented organic matter') uses facultative anaerobic lactobacilli bacteria to convert sugar into lactic acid, which results in improved growth, yield, quality and protection of vegetables and crops (Dou et al. 2012). Most published studies (70%) reported a positive effect of such lactic fermented bokashi amendment on the growth of vegetables (Olle and Williams 2013), as well as on plant available nutrients (available P) and crop yield (Liu et al. 2012; Andreev et al. 2016). However, no systematic research exists on the agronomic effect of lactic fermented bokashi-biochar mixtures.

In the present study, three different processes of composting, both in the presence and absence of biochar were tested: 1) Conventional composting; 2) Aerobic composting and 3) Bokashi fermentation. Composts were added to soils alone (Comp), together with biochar but added separately (Comp+BC_{post-mix}), and together with biochar after co-composting such that biochar and compost were added together (Comp+BC_{co-comp}). The three methods differ from each other, as conventional composting (Comp.conv) does not involve turning the piles, while aerobic composting (Comp.aer) involves turning the piles and bokashi fermentation (Bok) is an anaerobic lacto-fermentation process in a closed environment. Little research exists (for example, the study from Glaser et al. (2015)) in which organic nutrient transformations techniques in the presence of biochar additives were investigated and their effects on soil fertility and plant growth were assessed. Using biochar as an additive during the composting process (co-composted) may increase the fertilizing efficiency of the nutrients. These co-composted organic amendments

were compared with inorganic amendments (both in presence and absence of biochar) and with compost alone. This paper aimed at demonstrating that subsistence farmers in tropical countries may improve their on-farm organic nutrient management to achieve fertilizer efficiencies comparable or even better than expensive, imported based mineral fertilizers.

Biochar used in this experiment was produced from the invasive, non-palatable feedstock, *Eupatorium*, ubiquitous in Africa (Mshandete and Parawira 2009) as well as South and East Asia (Liu et al. 2006). In the present study, we hypothesized that the biochar-compost mixtures, especially co-composted biochar, when compared with inorganic treatments and compost alone could 1) enhance soil available nutrients (mainly P and K); and 2) increase maize biomass growth as a result of the increased soil nutrient availability in this soil.

Materials and methods

Composting methods

The raw materials used for the composting process were green waste (mixed vegetable and *Eupatorium* waste in the ratio of 20:80, chopped to 3–5 cm length), cattle farmyard manure (FYM) and biochar (BC). Green waste was collected from agricultural farmland and manure from a cattle farm located at Pathik Foundation, Kathmandu Valley, Nepal. Biochar was produced from '*Eupatorium adenophorum*' feedstock using a flame curtain steel shielded soil pit 'Kon-Tiki' kiln with a pyrolysis temperature of 600–700°C (Cornelissen et al. 2016). The elemental content of *Eupatorium* was 42.9% C, 1.4% H and 1.5% N (Pandit et al. 2017). The biochar had a $\text{pH}_{\text{CaCl}_2}$ of 8.9, CEC of 121 $\text{cmol}_c \text{ kg}^{-1}$ (measured with 1 M NH_4NO_3 extraction) and an organic carbon content of 71.4% (Pandit et al. 2017).

Three composting methods were used to produce organic-based fertilizer formulations: 1) conventional composting (Comp.conv), 2) aerobic composting (Comp.aer) and 3) bokashi fermentation (Bok). For all three methods, raw materials (green waste:manure ratio of approx. 1:1.5 w/w wet weight) were mixed thoroughly in the absence and presence of biochar (10% vol.). Addition of 10% biochar during composting or matured stored compost has been shown to be optimal for making biochar-based organic fertilizers (Kammann et al. 2016). During this process, 144 kg of chopped green waste and 224 kg manure were mixed to make homogenous mixtures and separated into two equal portions (184 kg each), after which 16 kg wet biochar was added to one portion (equivalent to 10% by volume, 6 kg dry biochar). The portion without biochar was separated into three heaps for conventional composting (46 kg, 16.5 kg dry weight), aerobic composting (92 kg, 33 kg dry weight) and bokashi fermentation (46 kg, 16.5 kg dry weight). The portion with biochar (co-composted) was also divided into three heaps with the same mass as for composting without biochar; conventional composting with biochar (Comp.conv+BC_{co-comp}), aerobic composting with biochar (Comp.aer+BC_{co-comp}) and bokashi biochar fermentation (Bok+BC_{co-comp}). Conventional co-compost, aerobic co-compost and bokashi co-compost heaps received 1.5 kg, 3 kg and 1.5 kg dry biochar, respectively, which was equivalent to 10% vol. biochar.

Both heaps for conventional composting (with and without biochar) were stored at the same location and the entire composting process was completed without turning the piles (Misra et al. 2003). Under aerobic composting, 5 kg of clay soil (wet weight) collected from a rice paddy field was added to both of the heaps (with and without biochar) and mixed thoroughly. Clay was added to improve water holding capacity, and especially to allow the formation of clay-humus complexes during composting which improves the compost quality and the stability of the exchangeable nutrients and increases CEC and microbial activity of the compost. Both aerobic composting heaps were kept in the shade of a shelter to provide protection from rainfall and to ensure optimum humidity conditions required for good quality compost. Aerobic compost was matured by manual turning the composting piles daily for the first three weeks, and every three d after that (Hagemann et al. 2018). For bokashi fermentation, raw materials from two heaps (with and without biochar) were placed on two separate plastic sheets in layers (six layers in total for each heap). Thus, each

layer of bokashi and bokashi-biochar fermentation received 2.75 kg and 3 kg of raw materials (dry weight equivalent). Before adding each of the next layers, 150 g sugar (900 g of sugar in total) was applied along with 100 mL spray of diluted fermentative liquid (1:20 parts; 600 mL in total) followed by the compaction of each layer with the help of a ram. The fermentative liquid was prepared in 1.5 L bottles where 300 mL fermentative liquid from the previous batch and 30 g fresh mixed leaves were added to 1 L of water. This starter blend was anaerobically fermented for 10 d. Both plastic sheets were entirely closed and soil was placed on the top of the sheets to ensure anaerobic conditions. Bokashi fermentation involves lacto-bacilli activity under the anaerobic condition to break down the organic substrates (Andreev et al. 2016). All three composting processes lasted for 80 d (11th July – 29 September 2016).

Physicochemical characterization of compost

Monitoring during composting

During the composting process, moisture content (% vol.), temperature ($^{\circ}\text{C}$) and redox potential (mV) were measured every 7 d until compost maturation. pH of compost and co-composted BC-compost from conventional and aerobic composting piles were measured at 40 d and 80 d. For practical reasons, the anaerobically packed bokashi fermentation systems, moisture content, temperature, Eh and pH were monitored only once, after harvesting of the product (80 d). Moisture content (% vol.) was measured (three measurements per pile) by a hand-held Time-Domain Reflectometer (TDR; SM150 soil moisture sensor, Delta T devices Ltd, Burwell, Cambridge, England). Composting piles were watered when they had moisture contents less than 40% (measured with TDR), to prevent a decrease of microbial activity. Compost pH was measured in 0.01 M CaCl_2 solution (1:5 solid-water ratio) with a WTW pH 320 device. Eh (mV) was measured with WTW equipment with an AgCl reference electrode (combined 3 M AgCl electrode) and corrected to standard hydrogen electrode (SHE) as a function of temperature. The temperature of composting piles was recorded with temperature sensor rods.

Compost characterization

Compost and co-composted BC-compost samples were collected (after compost harvest) randomly from different portions within each heap for chemical analysis. Samples were oven dried at 40°C 3 d and passed through a 2 mm sieve prior to analysis. For total P and K analysis, compost samples (0.25 g) were first decomposed in ultrapure nitric acid using an ultraclave at 260°C and at a pressure of about 50 bars. After decomposition, samples were diluted to 50 mL with deionized water and analyzed through microwave assisted nitrogen plasma instrument (Agilent 4200) via selective atomic lines (213.618 nm for P and 769.897 nm for K). For NO_3^- and NH_4^+ analysis, samples were extracted with 2 M KCl solution (5 g dry compost added to 25 mL of 2 M KCl solution; 1:5 solid to solution ratio). This solution was shaken (100 rpm) for 30 min, filtered through pre-washed blue ribbon filters (Whatman 589/3), and was introduced in a flow injection system (FIA star 5000) for analysis. Available (ammonium lactate extractable) phosphorus (P-AL) was measured according to Krogstad et al. (2008). In this process, 2 g dry compost was added to 40 mL ammonium lactate solution, filtered (Whatman filter paper) and diluted 10 times. Ascorbic acid (0.4 mL) and molybdenum reagent (0.4 mL) were added to the diluted samples and standards. Measurements were done using spectrophotometry (Pandit et al. 2017).

Greenhouse experiment design

A pot trial was carried out under greenhouse conditions for 55 d (from 12th October–8 December 2016) at Matatirtha, Kathmandu, Nepal (N $27^{\circ} 41' 51''$, E $85^{\circ} 14' 0''$, altitude 1520 m). Average temperature recorded inside the greenhouse during the trial period was 23.5°C (average minimum 15°C and average maximum 32°C , $n = 50$). Nursery plant pots (top, middle and bottom diameter; 24 cm, 19 cm and 12 cm, respectively, and 20 cm high) with approx. 6 L volume was filled

with 3 kg of air-dried silty loam (Inceptisol) that was collected from arable soil, Rasuwa district, Nepal (27°, 59,479' N and 85°, 11,987' E), as described in Pandit et al. (2018a). No crowding of roots inside the pots was observed, in line with field experiments where root-to-shoot ratios of maize plants in similar soils were in the order of 2–5%, and root systems weighed in the order of 10–20 g (Abiven et al. 2015).

The experiment consisted of 88 pots in a completely randomized design and included 22 treatments with four replications each ($n = 4$).

Three types of compost, i.e., conventional compost, aerobic compost and bokashi fermentation with premixed or co-composted biochar (Comp.conv+BC_{co-comp}, Comp.aer+BC_{co-comp} and Bok+BC_{co-comp}), post-mixed biochar (Comp.conv+BC_{post-mix}, Comp.aer+BC_{post-mix} and Bok+BC_{post-mix}) and without biochar (Comp.conv, Comp.aer and Bok) were applied in two different dosages (40 g per pot and 120 g per pot equivalent to 20 t ha⁻¹ and 60 t ha⁻¹ respectively) resulting in 18 treatments. In addition to these, four additional treatments were tested; (1) NPK equivalent to nutrient content in 20 t ha⁻¹ compost (0.12 g N, 0.06 g P₂O₅ and 0.24 g K₂O), (2) NPK equivalent to nutrient content in 60 t ha⁻¹ compost (0.36 g N, 0.18 g P₂O₅ and 0.72 g K₂O), (3) NPK equivalent to nutrient content in 20 t ha⁻¹ compost + 3 g biochar and (4) NPK equivalent to nutrient content in 60 t ha⁻¹ compost + 9 g biochar). By assuming a 15% N availability, 30% P and K availability in the compost (Kammann et al. 2016), the amount of NPK content in 20 t ha⁻¹ and 60 t ha⁻¹ compost was calculated. Mineral nutrient NPK was applied in the form of Urea for N, orthophosphate for P₂O₅ and murate of potash for K₂O.

After mixing all the organic and inorganic amendments thoroughly in the respective treatment, three maize seeds (Manakamana-4 variety) were sown 2 cm below the soil surface in each pot. Upon germination and emergence of two leaves (14 d), the smaller and least robust plants, selected based on visual observation, were removed from the pots to leave one plant for the experimental duration. All the pots were irrigated daily with 140 mL water pot⁻¹ day⁻¹ until second leaf emergence (14 d) after which the pots were irrigated every five d at 700 mL water pot⁻¹ until harvest. These watering rates are representative of the growth season in Nepal (Pandit et al. 2018a). Pots were rotated every four days until harvest to ensure homogeneity of the treatments (exposure to sunlight, shade, humidity, etc.). Weeding was carried out twice (30 d and 42 d) during the experiment.

In-situ soil physicochemical analysis

Soil moisture content (% vol.) was measured every five days until harvest (55 d) following exactly the same procedure as described in Pandit et al. (2018a). Soil redox potential (Eh) was measured at 30 d and 55 d with the same device used for in-situ compost (Eh) measurement. Measured Eh (mV) was corrected to SHE as a function of temperature.

Plant harvest and soil analysis

Maize plants were harvested on 55 d and fresh weight of above ground biomass (AGB) was measured immediately after harvest. Maize AGB was oven dried at 70 °C for 24 h to calculate the dry weight (g).

Soil samples were collected at 1 d, 30 d and 55 d of pot trial and analyzed for soil pH (0.01 M CaCl₂ solution at a 1:5 solid to water ratio). For all other soil tests, soil samples were collected after maize plants were harvested. Soil from all individual pots was collected to make a bulk composite sample for each of the 22 treatments. Dried (105 °C; 12 h) and sieved (2 mm) soil samples were analyzed for CEC, exchangeable acidity (H⁺) and plant available phosphorous (P-AL) following Pandit et al. (2018b). For soil CEC measurement, samples were extracted with 1 M NH₄NO₃ at pH 7 and the exchangeable cation concentrations were determined using ICP-OES. Exchangeable acidity (H⁺) was determined by titration the extract with 0.02 M NaOH to pH 7. Plant available phosphorous

(P-AL) was measured similar to the compost analysis using the ammonium lactate method (Krogstad et al. 2008). For soil NO_3^- and NH_4^+ measurement, fresh samples were extracted within 12 h with 2M KCl solution (20 g dry soil added to 50 mL of 2 M KCl solution and measured with a flow injection system (FIA star 5000), similar to the compost analysis.

Statistical analysis

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, respectively. One factor ANOVA was used to assess the effect of three different processes of composting (with and without BC) on composting quality (aeration, moisture content, temperature) and the available nutrients in the matured compost. Likewise, one factor ANOVA was used to assess the effect of various organic (compost) and inorganic amendments (NPK) with and without biochar (categorical explanatory variable) applied at two different dosages (20 t ha^{-1} and 60 t ha^{-1}) on soil physicochemical properties and maize biomass production (response dependent variable). REG-WQ (Ryan/Einot and Gabriel/Welsch + test procedure) post hoc test ($p = 0.05$) was used to evaluate the significant differences between various treatment means. The differences between treatments were significant at $p < 0.05$, unless otherwise stated. A linear regression model was used to assess the correlation between maize biomass production and the various soil parameters (pH, Eh, nitrate, ammonium, P-AL, K^+ , Ca^+ and Mg^+).

Results

Composting conditions

The average moisture content was 5–15% higher for biochar-amended composts than for non-biochar composts for all three composting systems throughout the composting period (Figure 1(a)). Recorded temperatures were in the range of the mesophilic phase (below 40°C) but a thermophilic phase (above 40°C) was not reached, neither for compost nor for biochar co-compost in the conventional and aerobic composting piles (Figure 1(b)). Similar to moisture content, average Eh was around 50 mV higher for biochar-amended composts than for non-biochar composting ones (Figure 1(c)). The pH of composting piles measured at 40 d and 80 d did not show significant variation; therefore, the values were averaged to give one reading for each of the compost and co-composted piles. Aerobic co-composted biochar (Comp.aer+BCco-comp) had the highest pH (7.9 ± 0.1) and bokashi fermentation (Bok) showed the lowest pH (pH 4.89 ± 0.04) (Table 1). By contrast, bokashi in the presence of biochar (Bok+BCco-comp) was neutral (pH 7.20 ± 0.02). Previous work (Probst et al. 2015) has demonstrated that lactic acid fermentation occurred at neutral pH.

Nutrient content of composts and co-composted biochar-composts

Total K and P and available P were higher for bokashi fermentation (Bok and Bok+BC_{co-comp}) compared to the other two composting processes (Table 1). Inorganic N contents (NO_3^- and NH_4^+) were observed to be higher for conventional (Comp.conv) and aerobic composts (Comp.aer) than bokashi fermentation (Bok). Bok+BC_{co-comp} fermentation substrate contained higher NO_3^- ($61.0 \pm 1.5 \text{ mg kg}^{-1}$) compared with bokashi fermentation in the absence of biochar ($32.01 \pm 0.08 \text{ mg kg}^{-1}$) (Table 1).

Biomass production

Bokashi applied at 60 t ha^{-1} in the presence (but not the absence) of biochar showed a strong positive effect on maize biomass production, especially after co-composting (Figure 2).

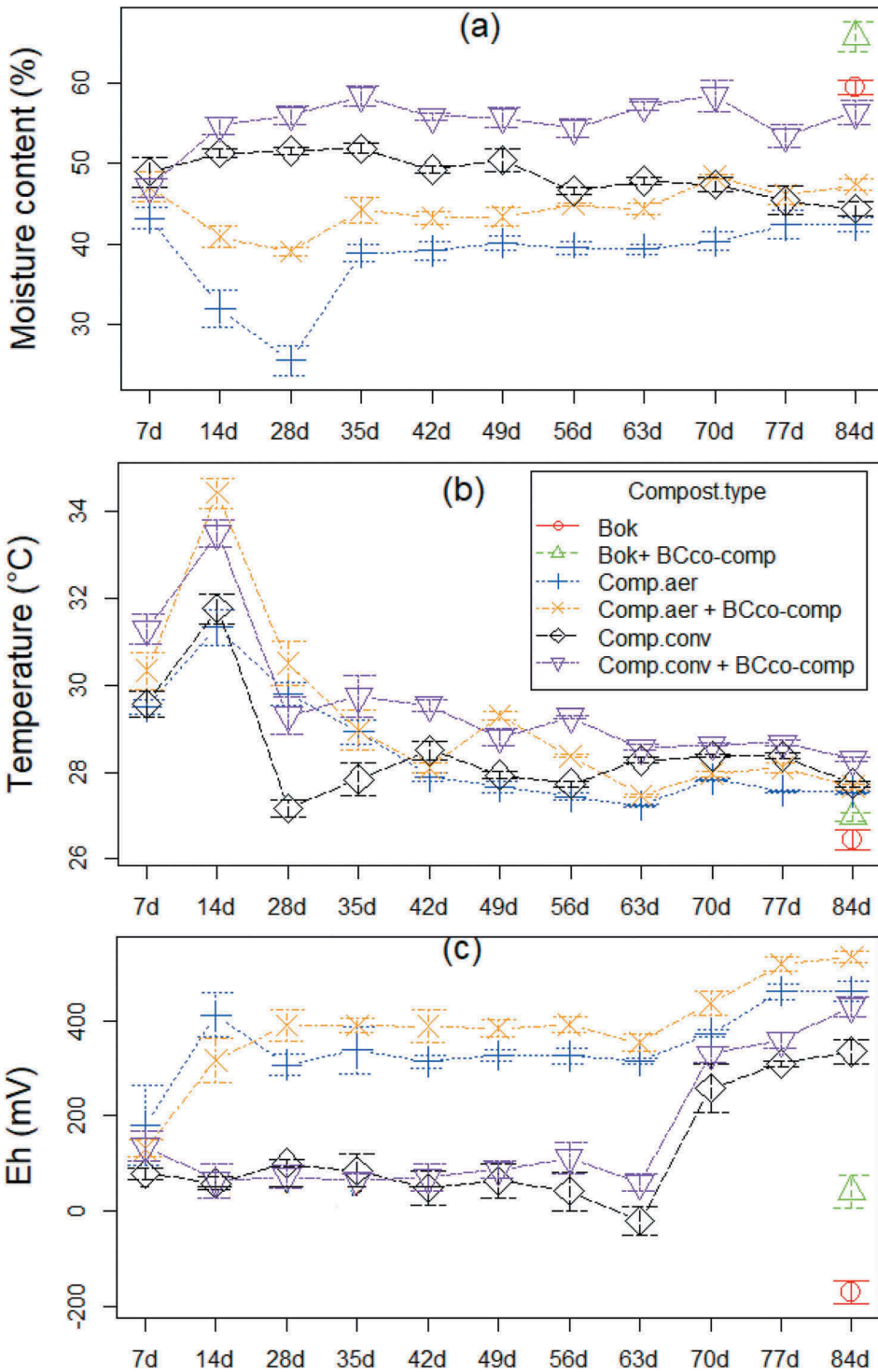


Figure 1. Average moisture content, temperature and Eh of different composting piles (y-axis) measured at every 7 d until compost harvest (x-axis), n = 3. Legend (compost types) of these three measurements is shown in Figure 1(b).

Table 1. Nutrient content and pH of different composts and co-composted biochar-composts (mean \pm sd).

Treatments	pH		Nutrient content of compost			
	$n = 2$	P (g kg^{-1}), $n = 3$	K (g kg^{-1}), $n = 3$	P-AL ^a (g kg^{-1}), $n = 2$	NO_3^- (mg kg^{-1}), $n = 2$	NH_4^+ (mg kg^{-1}), $n = 2$
Comp.conv	7.26 \pm 0.03	1.7 \pm 0.0	19.0 \pm 0.0	0.84 \pm 0.01	72.5 \pm 3.5	60.0 \pm 0.0
Comp.conv + BCco-comp	7.90 \pm 0.10	1.7 \pm 0.2	17.3 \pm 1.5	0.86 \pm 0.01	85.0 \pm 0.0	37.3 \pm 0.4
Comp.aer	7.33 \pm 0.35	3.4 \pm 0.3	20.3 \pm 1.2	2.16 \pm 0.01	925 \pm 35.4	16.5 \pm 0.7
Comp.aer + BCco-comp	7.90 \pm 0.11	3.9 \pm 0.1	21.3 \pm 3.2	2.02 \pm 0.01	185 \pm 14.1	18.5 \pm 0.7
Bok	4.89 \pm 0.04	6.2 \pm 0.1	25.3 \pm 1.2	4.22 \pm 0.00	32.0 \pm 0.1	7.9 \pm 0.6
Bok + BCco-comp	7.23 \pm 0.06	6.3 \pm 0.1	28.0 \pm 1.0	4.25 \pm 0.01	61.0 \pm 1.5	9.7 \pm 1.9

^aPlant available phosphorus

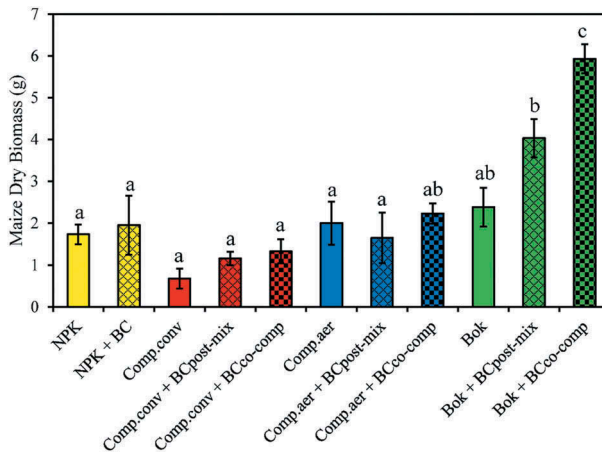


Figure 2. Effect of various organic and inorganic amendments in the presence and absence of biochar applied at the rate of 60 t ha^{-1} composts on maize biomass production (mean \pm SE, $n = 4$). Different letters above a bar of each treatment represent significant differences between various treatments following one way-ANOVA (post-hoc-REG-WQ test, $p < 0.05$).

Bok+BC_{co-comp} significantly increased biomass production per pot (5.93 \pm 0.71 g) by 243%, 204% and 149% compared with NPK (1.73 \pm 0.57 g), NPK+BC (1.95 \pm 1.42 g) and bokashi without biochar (2.38 \pm 0.46 g) respectively (Figure 2). Bok+BC_{post-mix} also showed increased biomass production (4.03 \pm 0.93 g) compared with NPK and NPK+BC, but the effect was less pronounced so (+132% and +106%, respectively; Figure 2). Compost and BC-compost produced from conventional and aerobic systems showed no significantly different biomass production from NPK (control) and NPK +BC (Figure 2). None of the composts and/or co-composted compost-biochar formulations showed any significant differences from NPK and NPK+BC treatments at the application rate of 20 t ha^{-1} (results not shown).

Soil properties after the trial

Available P in post-trial soil was significantly higher for biochar-compost mixtures (both co-compost and post-mix BC-compost applied at 60 t ha^{-1}) produced using all three composting methods (44 to 105 mg kg^{-1}) when compared with NPK and NPK+BC treatments (34 and 38 mg kg^{-1} respectively) (Table 2). Much higher P-AL was observed for Bok+BC_{co-comp} (105 mg kg^{-1}) than for all other organic amendments with and without biochar. No differences between Bok+BC_{co-comp} and other organic and inorganic amendments were observed on soil P-AL when applied at the rate of 20 t ha^{-1} (results not shown). Soil NO_3^- was



Table 2. Effect of organic amendments (compost and co-compost) mixed with and without biochar and applied at 60 t ha⁻¹ on soil physicochemical properties. Different letters within each column denote significant differences between treatments on soil properties following one-way ANOVA (REG-WQ test, $p < 0.05$).

Treatments	In-situ soil properties				Ex-situ soil characterization, n = 3									
	Moisture		Eh		pH									
	(%), n = 32	(mV), n = 8	n = 12								BS (%)		NH ₄ ⁺ mg kg ⁻¹	
				Mg ²⁺	Ca ²⁺	K ⁺	Al ³⁺	H ⁺	CEC ^a			P-AL mg kg ⁻¹	NO ₃ -N mg kg ⁻¹	NH ₄ ⁺ mg kg ⁻¹
NPK	8 ± 1a	540 ± 13c	5.4 ± 0.3a	1.2 ± 0.01a	4.0 ± 0.1a	0.7 ± 0.04c	0.21 ± 0.01c	1.69 ± 0.34d	7.8 ± 0.2a	76 ± 3a	34.8 ± 4.7ab	5.1 ± 2.1ab	10.4 ± 0.5b	
NPK + BC ^b	11 ± 1b	560 ± 10de	5.9 ± 0.1b	1.2 ± 0.04a	4.5 ± 0.2b	1.0 ± 0.03e	0.07 ± 0.01b	0.00 ± 0.00a	7.8 ± 0.2a	99 ± 0d	38.3 ± 2.3b	7.9 ± 0.9c	6.1 ± 3.2a	
Comp.conv	11 ± 1b	524 ± 13bc	6.3 ± 0.1bc	1.8 ± 0.04b	5.7 ± 0.2c	0.7 ± 0.01c	0.00 ± 0.00a	0.10 ± 0.18a	8.4 ± 0.2b	98 ± 2cd	43.0 ± 2.9bc	6.7 ± 4.7abc	8.0 ± 3.4ab	
Comp.conv+BC ^{post-mix}	11 ± 1b	540 ± 11c	6.6 ± 0.1d	1.8 ± 0.11b	5.6 ± 0.2c	0.9 ± 0.08de	0.00 ± 0.00a	0.70 ± 0.39bc	9.0 ± 0.7bc	92 ± 4b	44.0 ± 0.5c	4.8 ± 1.0ab	6.5 ± 3.4ab	
Comp.conv+BC ^{co-comp}	11 ± 1b	552 ± 14cd	6.4 ± 0.1cd	1.9 ± 0.08bc	6.0 ± 0.2d	0.6 ± 0.02b	0.02 ± 0.03ab	0.00 ± 0.00a	8.6 ± 0.4bc	99 ± 0d	52.7 ± 7.0cde	4.6 ± 2.6ab	8.7 ± 4.0ab	
Comp.aer	11 ± 2b	503 ± 7a	6.5 ± 0.1cd	2.0 ± 0.04c	6.2 ± 0.2d	0.5 ± 0.03a	0.00 ± 0.00a	0.58 ± 0.09b	9.3 ± 0.3c	93 ± 1b	32.1 ± 1.4a	4.0 ± 0.6a	8.2 ± 3.0ab	
Comp.aer+BC ^{post-mix}	13 ± 2b	509 ± 11ab	6.8 ± 0.1d	2.0 ± 0.00c	6.3 ± 0.0d	1.0 ± 0.06de	0.00 ± 0.00a	0.89 ± 0.17c	10.3 ± 0.1d	90 ± 1b	49.0 ± 2.2d	7.9 ± 4.6bc	9.0 ± 2.8ab	
Comp.aer+BC ^{co-comp}	12 ± 2b	579 ± 8e	6.8 ± 0.1d	2.0 ± 0.12c	6.2 ± 0.5cd	0.9 ± 0.02d	0.00 ± 0.00a	1.32 ± 0.21d	10.5 ± 0.4d	87 ± 2b	55.1 ± 2.1e	5.0 ± 2.2ab	7.9 ± 2.7ab	
Bok	11 ± 1b	503 ± 10ab	6.1 ± 0.1b	1.6 ± 0.08b	4.9 ± 0.2b	0.9 ± 0.08de	0.00 ± 0.00a	2.35 ± 0.34e	9.8 ± 0.6cd	76 ± 2a	38.4 ± 1.4b	5.2 ± 0.3b	9.0 ± 5.1ab	
Bok ₁ -BC ^{post-mix}	16 ± 2c	558 ± 10de	6.6 ± 0.1d	2.1 ± 0.04c	6.1 ± 0.2cd	1.3 ± 0.03f	0.01 ± 0.00b	0.51 ± 0.18b	10.2 ± 0.3d	94 ± 2bc	57.7 ± 2.3e	6.4 ± 1.0bc	10.5 ± 2.2ab	
Bok ₁ -BC ^{co-comp}	17 ± 2c	546 ± 11c	6.7 ± 0.1d	2.5 ± 0.11d	7.3 ± 0.6e	1.7 ± 0.12g	0.00 ± 0.00a	0.00 ± 0.00a	12.0 ± 0.9e	95 ± 0c	105.1 ± 2.8f	8.7 ± 2.6c	10.7 ± 2.6ab	

^a CEC measured as the sum of exchangeable cations and extractable acidity (H⁺); Na⁺ was also included for the CEC calculation (not shown in the table).

^b Biochar applied at the rate of 9 t ha⁻¹.

significantly increased upon amendment with Bok+BC_{co-comp} and Bok+BC_{post-mix} compared with bokashi without biochar applied at 60 t ha⁻¹ (Table 2).

Soil CEC was significantly increased for all biochar-composts mixtures applied at 60 t ha⁻¹ (8.4 to 12 cmol_c kg⁻¹) compared to NPK with and without biochar (7.8 cmol_c kg⁻¹) (Table 2). All compost and BC-composts showed higher pH and lower amounts of exchangeable Al³⁺ compared with NPK treatment (Table 2). However, even the Al³⁺ in the NPK treatment was below levels where effects on plant roots can be expected (around 0.2 cmol_c kg⁻¹) (De Wit et al. 2001).

The average soil moisture content (% vol.) measured by daily TDR (n = 32) was significantly increased for Bok+BC_{co-comp} (17 ± 2%) and Bok+BC_{post-mix} (16 ± 2%) compared with other organic and inorganic amendment when applied at 60 t ha⁻¹ (Table 2) but not at 20 t ha⁻¹ (results not shown).

Discussion

Composting conditions

The addition of biochar during aerobic composting under the shelter resulted in optimal moisture content (> 40% vol. required for effective microbial activity) for longer periods compared with the non-biochar aerobic piles (Figure 1(a)). This was mainly due to increased water-holding capacity resulting from the amendment of biochar, and supports previous studies (Kammann et al. 2016; Pandit et al. 2018a). For conventional and bokashi fermentation piles, higher moisture levels were also observed for biochar amended composts (Figure 1(a)). Similarly, Eh was higher for biochar-amended compost throughout the composting period compared to non-biochar composting piles, possibly due to the higher porosity of biochar that maintains the higher oxygen level for longer periods (Kammann et al. 2016). However, the measured values of Eh were slightly lower (below 500 mV) (Figure 1(c)) than is normally expected following biochar addition (Eh > 500 mV), but were still in the range required for good soil quality (Husson 2013). Among all compost and co-composted types, bokashi fermented compost and co-compost had a significantly higher amount of available nutrients (P-AL, K and NO₃⁻) (Table 1). In accordance with this, Boechat et al. (2013) reported accelerated organic matter degradation upon bokashi fermentation that enhanced available mineral nutrients in the system, and thus could reduce the requirement of nutrient supplements (John et al. 2007). Bokashi fermentation in the presence of biochar (Bok+BC_{co-comp}) had higher amounts of NO₃⁻ than bokashi without biochar (Bok) (Table 1), which could possibly be explained by the higher Eh in Bok+BC_{co-comp}.

Soil physicochemical properties and available nutrients

In addition to improved soil CEC and base cations (K⁺, Ca²⁺, Mg²⁺), soil P-AL was found to be highest for the co-composted biochar from bokashi fermentation (60 t ha⁻¹) (Table 2). Indeed, the ratio of available P to total P (P-AL/total P) where total P was equal for all bokashi fermentation with and without biochar (Table 1), follows the order: Bok < Bok+BC_{post-mix} < Bok+BC_{co-comp} (Figure 3). Co-composted biochar from aerobic compost (Comp.aer+BC_{co-comp}) also had higher soil P-AL and K⁺ contents compared with aerobic compost without biochar (Comp.aer) and NPK treatments, which supports the earlier observations of increased soil nutrients availability (available P and K) following co-composting with biochar (Prost et al. 2013; Glaser et al. 2015; Kammann et al. 2015). However, the availability of nutrients in the soil with aerobic and conventional compost in the presence of biochar were far less than those observed for bokashi fermented biochar amendments. This may be due to the lactobacilli amended during bokashi fermentation that enhanced microbial organic degradation which in turn increased nutrient availability in the soil system (Boechat et al. 2013). This was not found in the study conducted by Glaser et al. (2015). In addition, during bokashi fermentation, most of the nutrients are preserved in the hermetic fermentation pack, unlike conventional (open condition) and aerobic composting (piles

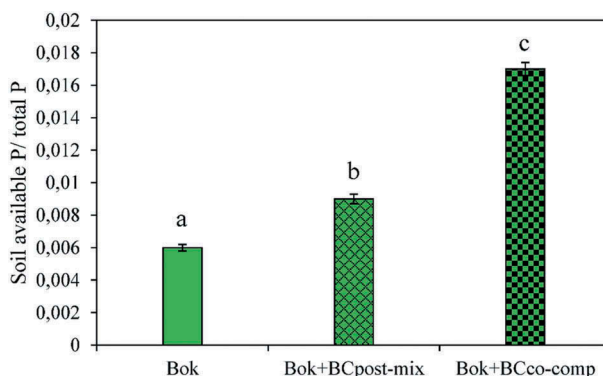


Figure 3. Ratio of soil available P and total P (P-AL/tot P) for bokashi fermentation in the absence and presence of biochar; mean \pm sd, $n = 3$.

sheltered with rooftop but open from side) that were subjected to nutrient leaching and elemental losses in the rainy season (aerobic piles mainly affected by lateral rainfall) during which composting took place (Hagemann et al. 2018). Beneficial effects of co-composted biochar on soil physicochemical properties and available nutrients (P-AL, K^+ , NO_3^-) have previously been reported by Agegnehu et al. (2016). Increased nutrient retention could be due to the formation of organic coating in co-composted biochar, which entrap or adsorb dissolved nutrients in the system (Kammann et al. 2015; Hagemann et al. 2017; Joseph et al. 2017).

Maize biomass production

In this study, various organic amendments (with and without biochar) did not demonstrate significant effects on maize biomass production with the exception of bokashi-biochar, where positive effects were especially prominent after co-composting (Figure 2). Glaser et al. (2015) found lower maize yields on a sandy Inceptisol under temperate field conditions after application of 40 t ha⁻¹ fermented biochar digestate when compared to non-fermented biochar digestate. In accordance with this, Andreev et al. (2016) reported significantly higher maize height following the amendment of bokashi fermented-biochar mixtures compared to a control, mineral fertilizers and other organic amendments (stored feces, stored cattle urine and stored urine) in field trials in loamy eroded soils. This reflects the positive effect of co-composted biochar-bokashi (Bok+BC_{co-comp}), which has significant growth in promoting features compared with biochar and compost alone (Kammann et al. 2016). This is possibly due to the activity of lactobacilli in bokashi fermentation that increases the amount of available nutrients which results in improved crop growth, quality and yield (Dou et al. 2012). This may be due to the higher amount of labile carbon (molasses) and nutrients most probably phosphorous in bokashi fermentation (Table 1) (Mayer et al. 2010). In accordance with this, Agegnehu et al. (2016) reported beneficial effects following the amendment of co-composted biochar on soil available nutrients, and a subsequent positive effect on crop growth and development.

Among various soil factors explored as a function of organic and inorganic amendments (60 t ha⁻¹) on maize biomass production, soil P-AL ($R^2 = 0.55$) and exchangeable base cations such as K^+ ($R^2 = 0.64$), Ca^{2+} ($R^2 = 0.35$) and Mg^{2+} ($R^2 = 0.36$) stood out and showed significant positive relationships ($p < 0.001$) with maize biomass production (Figure 4). However, statistically significant positive relationship between these soil parameters (P-AL, K^+ , Ca^{2+} and Mg^{2+}) and maize biomass were only observed when bokashi/biochar mixtures were included (results not shown). In addition, other soil factors such as soil NO_3^- , NH_4^+ , pH and Eh did not show a significant positive correlation with biomass production (results not shown). Measured mineral N (NO_3^- , NH_4^+) at the end of the

experiment could provide an indicator for available N and its relationship with maize biomass production. However, in our previous study with similar soil and crop under greenhouse conditions available mineral N measured via in-situ plant root simulators (nutrient supply rates with cation and anion probes buried in soil) was not correlated with maize biomass production, illustrating no effect of soil available N on maize biomass in this soil. The relationship between soil moisture content and maize biomass was not investigated, as the measured moisture content (Table 2) was relatively low for all the treatments including bokashi-biochar mixtures (ranging from 8% to 17% vol.), and this variable was not considered as a potential soil factor for improved crop growth. Thus, the relatively high maize biomass production (at least double that of all other additions; Figure 2) of the co-composted bokashi-biochar formulation can possibly be explained by higher soil available nutrients such as P-AL, K^+ , Ca^{2+} and Mg^{2+} in this soil (Table 2, Figure 4). Optimal maize growth requires P-AL to be in the range of 50–80 $mg\ kg^{-1}$ (Krogstad et al. 2008). Most of the organic amendment (including co-composted biochar from aerobic and conventional compost) and inorganic amendments used in this work had soil P-AL $< 55\ mg\ kg^{-1}$, with the exception of Bok-BC_{co-comp} ($> 70\ mg\ kg^{-1}$), providing a possible explanation for the superior effects on crop growth that were observed for bokashi fermentation in presence of biochar (Table 2). Indeed, P deficiency symptoms were observed for many of the treatments including bokashi without biochar but not for bokashi-biochar formulations. In our previous pot trial with the same soil and crop type, P-AL was one of the most important growth limiting factors, and it was effectively alleviated upon

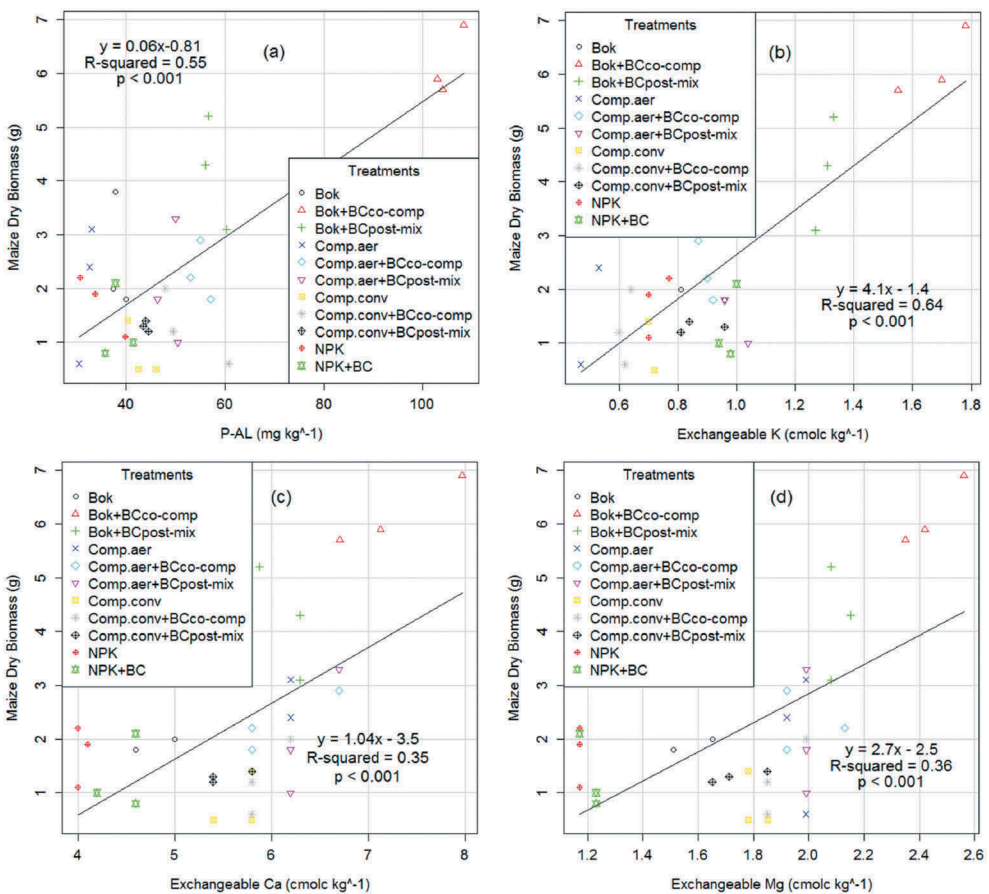


Figure 4. Relationship between P-AL and exchangeable base cations (K^+ , Ca^{2+} and Mg^{2+}) with maize biomass for various organic and inorganic treatments applied at $60\ t\ ha^{-1}$ of composts.

biochar amendment (increased P-AL from 11 mg kg⁻¹ at control to 84 mg kg⁻¹ at 2% w:w biochar addition). Furthermore, Bok+BC_{co-comp} also improved soil CEC mainly through increased exchangeable base cations such as K⁺, Ca²⁺ and Mg²⁺ (Table 2), which all contributed to the beneficial effect observed for biomass production (Figure 4). There are many previous studies that have observed that the amendment of biochar results in higher amounts of exchangeable base cations especially K⁺. These studies have concluded that the effects resulted in positive effects on crop production (Yamato et al. 2006; Martinsen et al. 2014; Agegnehu et al. 2016).

In addition, the reduced Eh of Bok-BC_{co-comp} (-71.31 ± 59.00 mV) amended to oxidize soil (> 400 mV) could lead to improvements of the soil-plant-microorganism system (Husson 2013) and thus a concurrent increase in biomass production. A factor contributing to the lack of positive agronomic effects of conventional and aerobic compost/biochar formulations may be that a thermophilic phase was not reached, with temperatures in the range of 60–70°C. The reason for this was possible that the compost piles used here were of a relatively small size.

In conclusion, hypothesis 1 that biochar-compost formulations could enhance soil available nutrients (mainly P and K) was accepted for aerobic and bokashi co-composting but rejected with regard to conventional co-composting. Hypothesis 2 that maize biomass growth would be increased as a result of this increased soil nutrient availability was only accepted for bokashi-biochar mixtures especially co-composted biochar-bokashi formulations. Hypothesis 2 was rejected for conventional and aerobic biochar-compost formulations.

A possible limitation of bokashi-biochar co-composting formulations could be that they were only effective at high compost addition rates of 60 t ha⁻¹, but not at usual compost dosages of 20 t ha⁻¹. More work is needed to find out whether the positive effect of adding bokashi-biochar formulations encompasses many soil types, or whether the effect was specific for the presently studied oxidized Inceptisol, where a high dosage was needed to improve the crop growth. The results shown here for maize may not be fully representative for other plants, and may vary with soil type and required available nutrient for proper growth and development. The improved crop growth for bokashi fermentation in the presence of biochar was most probably explained by increased nutrient availability, most notably P (reaching acceptable plant available P levels), possibly mediated by lactobacilli which can further increase plant nutrient availability and organic matter turnover. However, further long-term studies are needed to prove and reveal the effect of bokashi-biochar formulations (lactobacilli activity) on organic carbon degradation and bioavailable nutrients. Other effects of the lactic acid bacteria, such as on pathogens and other soil biota, were not studied here, and should be focused on in subsequent work. The present study investigated effects in related to a limited range of soil physical and chemical parameters, but detailed microbiological and spectroscopic studies are needed to mechanistically unravel the effects of bokashi-biochar formulations.

Implications and economic feasibility

We tested the economic feasibility of adding 60 t ha⁻¹ biochar/compost formulation to maize agriculture in Nepal. 60 t ha⁻¹ composted biochar contained 10 t ha⁻¹ biochar, 20 t ha⁻¹ green waste (4 t ha⁻¹ vegetable waste and 16 t ha⁻¹ Eupatorium weed) and 30 t ha⁻¹ cattle manure. Cost for these materials is USD 250 for 10 t biochar (Schmidt et al. 2015; Pandit et al. 2018b), USD 40 for 4 t vegetable waste, USD 320 for 16 t Eupatorium weed (cost of chopping), and USD 180 for 30 t cattle manure. Thus, total cost to produce 60 t ha⁻¹ co-composted biochar is 790 USD.

The benefit from maize production being 243% higher with 60 t ha⁻¹ co-composted biochar would imply a maize production increase of around 6 t ha⁻¹, worth USD 1800. Thus, a net benefit of USD 1010 (USD 1800 – USD 790) per hectare of maize land can be made through the addition of 60 t ha⁻¹ of co-composted biochar.

Disclosure statement

No potential conflict of interest was reported by the authors.

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