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**Biochar as soil amendment: Impact on chemical properties and corn nutrient uptake
in a Podzol**

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Syuhada et al. Biochar as soil amendment to a sandy Podzol. A study was conducted to investigate the impact of biochar amendment on chemical properties and corn nutrient uptake in a sandy Podzol soil. Four rates of biochar (0, 5, 10, and 15 g kg⁻¹) and two rates of inorganic fertilizer (0, and local recommendation rate for corn) were randomly applied to a completely randomized design with four replicates. Corn was grown for 45 days in a glasshouse using sandy Podzol. The increase in pH of the soil was concomitant with decrease of exchangeable Al. The fertilized soil significantly increased total N with concomitant decrease in soil pH due to N nitrification. Positive changes did occur in the soil due to biochar application, leading to significant increase in dry matter yield and corn height. Corn N and K uptakes were significantly increased by the addition of biochar, but the same was not true for Ca and Mg. However, it was found that concentration of N, Ca and Mg in the corn tissue was still lower than their critical level. Our results demonstrate that application of biochar alone is not able to supply enough nutrients for the healthy growth of corn.

Keywords: Empty fruit bunch biochar, soil properties, corn, NPK fertilizer, Podzol

Soil organic carbon (SOC) is one of the most important sources and sink for nutrients and it plays a vital role in maintaining soil fertility and crop productivity (Díaz-Zorita et al. 2002; Lal 2004; Pan et al. 2009) especially in strongly weathered and nutrient-poor tropical soils. In most tropical environments where high annual rainfall distribution coupled with warm temperature, loss of SOC becomes a serious matter to combat. Under these conditions, soil fertility can be low due to rapid mineralization of SOC (Jenkinson and Ayanaba 1977 cited in Major et al. 2010) and further decreases of cation exchange capacity (CEC) which correlates to soil nutrient depletion. Soil fertility and nutrient contents can be successfully improved by using either organic or inorganic fertilizers (Mando et al. 2005; Topoliantz et al. 2005). As consequence of high rainfall, the efficiency of applied inorganic fertilizers is very low because leaching of mobile nutrients, such nitrate (NO_3^-) and potassium (K) from the topsoil is enhanced (Melgar et al. 1992; Cahn et al. 1993). Application of organic fertilizers or amendments such as compost, mulch or manure may be more appropriate for nutrient retention under such circumstances than mineral fertilizers due to gradual release of nutrient (Burger and Jackson 2003; Steiner et al. 2007). However, the benefits of organic fertilizers are generally short-lived under humid tropical conditions because they mineralize rapidly and may last only for a few growing seasons (Bol et al. 2000; Glaser et al. 2002, Diels et al. 2004). Therefore, the organic fertilizers or amendments have to be applied frequently to sustain the soil fertility and improve nutrients retention. Alternatively, the application of more stable compound such as biochar instead of easily degraded organic amendments seems to be a promising option to achieve the purposes.

The application of biochar onto soils is not a new concept. Charred organic materials have been added to soils in the Amazon Basin by pre-Columbian farmers who practiced a form

of 'slash and char' agriculture (Sombroek et al. 2003) between 500 to 2500 years ago (Lehmann and Rondon 2006). These productive soils are called "Terra Preta de Indio" soils and have been shown to have high cation exchange capacity, improved nutrient holding capacity and more neutral pH when compared to the surrounding soils (Mann 2002; Lehmann et al. 2003a; Lehmann 2007; Fowles 2007; Laird 2008). The high fertility level and high organic matter content in these anthropogenic soils have persisted even several thousand years after they were abandoned by the populations that caused their formation (Lehmann et al. 2003b). Therefore, addition of charring biomass or charcoal is believed to be responsible for the prolonged high level of organic matter in the "Terra Preta de Indio" soils.

To date, the use of biochar as an amendment on highly weathered tropical soils has been reported to bring about numerous other benefits such as increase in soil pH, addition of basic cations and micronutrients, improvement of CEC, gradual release of nutrients to the growing plant and improving water holding capacity (Glaser et al. 2002; Laird et al. 2010; Sohi et al. 2010; Van Zwieten et al. 2010). Favorable effects of biochar application on crop productivity also have been observed in prior studies (Glaser et al. 2002; Lehmann et al. 2003a; Chan et al. 2007; Rondon et al. 2007; Chan et al. 2008; Asai et al. 2009). However, the majority of research on the effects of biochar in tropical regions was mostly conducted on clayey soils with a wide range of mixing rates of different biochar types. Moreover, few research data have been published elucidating the possible effects of biochar on sandy soils and crop responses, such as sandy Podzols of the tropics. The main problems of these Podzols are perched water table due to present of hard spodic layer, low nutrient availability, low CEC and poor moisture retention. Hence, the soils are not well utilized for agricultural activities and sometimes are left barren. However, some Spodosols may not have perched water table and these soils resemble other

quartzipsamments soil such as tin- tailing. Therefore, management of Spodosols with too deep spodic horizon and do not have perched water table are just similar to the management of quartzipsamments. Application of charcoal has proven to improve the fertility of sandy Podzols found in Malaysia and consequently improve plant yield (Malisa et al. 2011). Biochar is a new form of charcoal that can be used to amend this poor sandy soil. It has the ability to retain nutrient and moisture (Glaser et al. 2001), thus improve in physico-chemical properties of soil and crop productivity after biochar addition is expected.

Recently, a company in Malaysia utilized biomass from oil palm empty fruit bunch (EFB) for biochar production as a way of disposing it without harming the environment (Kong et al. 2014). Production of EFB biochar and its utilization for agriculture is new in Malaysia. Hence, a study of its potential for enhancing physico-chemical properties of soil and the subsequent crop productivity is fully justified. Therefore, to address the knowledge gap, we investigated the effect of biochar amendment on soil chemical properties and corn nutrient uptake in a sandy Podzol soil in Malaysia.

MATERIALS AND METHODS

Soil and biochar used

A sandy Podzol soil was collected from forest plantation at FRIM Research Experimental Station, Setiu, Terengganu (5.54 N, 102.87 E). The area was planted with forest species known as Balau pasir (*Shorea palembanica*). The soil is Jambu Series according to the Malaysian system of soil classification (Paramanthan 1987). According to the Canadian Soil Classification

system (Soil Classification Working Group 1998), the soil is classified as Humo-Ferric Podzol (Typic Haplorthods according to Soil Taxonomy (Soil Survey Staff 2010)).

It was very sandy in nature with a sand fraction of more than 90 %. Podzol used for this study has spodic horizon > 97 cm which is too deep below the soil surface; hence, management of this soil is similar to quartzipsamments. Therefore, for the purposed of this study, only the topsoil (0 – 15 cm) was collected for the experiment. The soil sample was air-dried and sieved to < 2 mm prior to treatment. The collected topsoil had a pH of 4.71 in water, total carbon (C) of 5.6 g kg⁻¹, CEC of 1.05 cmol_c kg⁻¹, total nitrogen (N) of 0.2 g kg⁻¹, exchangeable K of 14.82 mg kg⁻¹, exchangeable calcium (Ca) of 80 mg kg⁻¹, exchangeable magnesium (Mg) of 1.32 mg kg⁻¹ and exchangeable aluminium (Al) of 29.61 mg kg⁻¹.

The biochar used in the experiment was obtained from a local producer in Malaysia, Nasmeh Technology Sdn Bhd. The feedstock of the biochar was oil palm EFB. It was produced via slow pyrolysis using medium thermal process (300 – 350° C). The particle size of the biochar used was 2 – 5 mm; therefore, in order to guarantee a more homogenous application, the biochar was ground and sieved to < 2 mm and thoroughly mix with the sandy soil for the experiment.

Experimental Design

The experiment was carried out in a glasshouse at Universiti Putra Malaysia, Serdang, Malaysia. A completely randomized design (CRD) with 4 replications was used in the experiment using Mas Madu corn cultivar (*Zea mays* L.) as the test crop. The treatment layout was a factorial combination of four different rates (0, 5, 10, and 15 g kg⁻¹) of biochar and two rates of inorganic

fertilizer (0 and recommended rate). The biochar rates were 0, 25, 50 and 75 g pot⁻¹, which corresponded to 0, 10, 20 and 30 t ha⁻¹, respectively. Pots were filled with 5 kg of air-dried soil and biochar was applied a week before planting corn. Two seeds of corn were sown in the pots and thinned to one corn plant per pot two weeks after planting. The recommended rates of inorganic fertilizers were applied as follows: (i) 0.63 g of inorganic fertilizer in the form of NPKMg (12:12:17:2) at equivalent rate of 250 kg ha⁻¹ was applied to each of the fertilized pots one day before and 30 days after planting (DAP); and (ii) 0.33 g of urea (equivalent to 130 kg ha⁻¹) was applied at 15 and 30 DAP. Corn plants were irrigated daily to approximately 3.5% (w/w) of water holding capacity (at 33 kPa field capacity) and all pots received the same amount of water every day. They were constantly monitored up to 45 DAP. Total duration of the study was 52 days, which included 7 days of incubating the soil with biochar prior to planting and 45 days of corn growth.

Plant, soil and biochar analyses

At harvest (45 DAP), the height of corn was measured and the aboveground biomass was sampled. The corn plants were washed with distilled water and oven-dried at 60°C to constant weight before weighing to determine the total dry matter (DM) production of aboveground part of the corn. Thereafter, aboveground corn biomass was ground (< 1 mm) using a MF10 basic microfine grinder (IKA-Werke, Staufen, Germany). Dried and ground samples were digested using a modified method of Wolf (1982) and concentrations of Ca, Mg, and K in corn tissue were analyzed by an Optima 8300 ICP-OES spectrometer (PerkinElmer, Waltham, MA, USA),

whereas N in the tissue was determined using a TruSpec CHN analyser (Leco, St. Joseph, MI, USA). Nutrient uptake was calculated by multiplying DM by nutrient concentrations.

After harvesting, soil from each pot was air-dried and passed through a 2-mm sieve. The following soil analyses were carried out: (i) soil pH was determined using 1:2.5 ratio of soil: water; (ii) exchangeable Al was extracted with 1 M KCl at 1:10 (soil/solution ratio) by shaking for 30 minutes; (iii) soil total C and total N were determined using a TruSpec CHN analyser (Leco, St. Joseph, MI, USA); (iv) CEC and exchangeable cations (K, Ca and Mg) were extracted with ammonium acetate buffered at pH 7 (Schollenberger and Simon 1945). The CEC was determined using an auto-analyser (QuikChem 8000 Series FIA+ System; Lachat Instruments, Loveland, CO, USA) and the exchangeable cations were measured using an Optima 8300 ICP-OES spectrometer (PerkinElmer, Waltham, MA, USA).

Biochar pH values were obtained using 1 g of biochar in 20 ml distilled water with a shaking time of 1.5 h to ensure sufficient equilibration between the solution and biochar surfaces (Rajkovich et al. 2012). Total nutrient concentrations in the biochar were determined following digestion with a microwave system (ETHOS 1, Milestones, Italy) (Claoston et al. 2014). In brief, 5 ml of concentrated HNO₃ was added to 0.20 g biochar in a digestion vessel. Then, the vessel was placed in the microwave and heated at 200°C for 30 min. After cooling to room temperature, the digested solution was filtered into a 100-mL volumetric flask and brought to volume with distilled water. The concentrations of K, Ca and Mg were then determined by inductively coupled optical emission spectroscopy (ICP-OES) (Optima 8300 ICP-OES, PerkinElmer, Waltham, MA, USA), whereas C and N concentrations were determined using TruSpec CHN analyser (Leco, St. Joseph, MI, USA). The CEC of the biochar was measured based on the method of Song and Guo (2011).

Statistical analysis

The Shapiro-Wilk normality test was applied to all data using the UNIVARIATE procedure in SAS before performing the analysis of variance (ANOVA). Eleven parameters which were soil total C, total N, exchangeable K and Mg, exchangeable Al, concentrations of Ca and N in corn tissue, DM, and nutrient uptake of Ca, Mg, and N were log-transformed using natural log to achieve normality and homogeneity of variance. Two-way ANOVA was conducted using PROC MIXED in SAS for each parameter measured with biochar rate, fertilizer treatment and their interactions as fixed effects. Tukey's Studentized Range test was used to distinguish the differences among treatment means. The significant effects of biochar rates on all parameters were subjected to orthogonal polynomial contrast analysis (PROC MIXED) and regression analysis (PROC REG). All statistical analyses for the transformed parameters were carried out on the transformed data. The treatment means and standard errors from the transformed data were used to plot the graphs for the transformed parameters. Finally, the back-transformed means (mean_{bt}) were calculated by exponentiating the transformed means ($e^{\text{mean}} - 1$). All statistical analyses were performed using SAS software package Version 9.1. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Biochar chemical properties

The EFB biochar was alkaline in nature with a pH of 9.73 (in water) probably due to the presence of ash produced during the pyrolysis process and this was consistent with the finding of

Rabileh et al. (2015) and Norazlina et al. (2014) which studied the same type of biochar. The biochar used also contain high concentration of K with low concentration of N, Ca, and Mg with the value of 69 g kg^{-1} , 11 g kg^{-1} , 6.3 g kg^{-1} and 5.3 g kg^{-1} for K, N, Ca and Mg, respectively. Production of this EFB biochar under low temperature resulted in high content of C (533 g kg^{-1}) with very high CEC value ($69.62 \text{ cmol}_c \text{ kg}^{-1}$) (Norazlina et al. 2014). These properties proved that EFB biochar has the potential as a good amendment to improve soil fertility and consequently improve crop growth. However, biochar has to be applied in combination with other organic matter source or inorganic fertilizer to function better and maximize its benefits (Steiner et al. 2008).

Effects of biochar application on soil chemical properties.

The effect of biochar rate was significant for all soil chemical properties, except N (Table 1). The effect of inorganic fertilizer was significant only for soil pH. There was a significant biochar rate x fertilizer interaction only for N.

Soil pH increased as a quadratic function ($r^2=0.81$, $p<0.001$) of biochar rate (Table 1; Fig. 1a). The maximum soil pH was achieved at 6.42 when biochar was applied at 14 g kg^{-1} (Fig. 1a). Uzoma et al (2011), Van Zwieten et al. (2010) and Chan et al. (2007) also observed an increase in soil pH after the application of biochar. The increase in pH due to biochar application in this study coincided with the significant linear decrease in exchangeable Al concentration with biochar rate (Table 1). Soil treated with biochar ($\text{mean}_{\text{bt}} = 15.14$, 8.72 , and 2.80 mg kg^{-1} for 5, 10 and 15 g kg^{-1} biochar, respectively) also had significantly lower exchangeable Al compared to control ($\text{mean}_{\text{bt}} = 38.22 \text{ mg kg}^{-1}$). Regression analysis showed that exchangeable Al decreased

linearly ($r^2=0.75$, $p<0.001$) by 8% when biochar rate increased by one g kg^{-1} (Fig. 1c). Soil application of biochar could reduce Al concentration either due to: (i) decrease in exchangeable Al through adsorption on the surfaces of the negatively-charged biochar or (ii) reduction of Al activity in soil solution through chelation with soluble organic compounds from the biochar (Hue et al. 1986; Butnan et al. 2015). The increase in soil pH due to biochar liming effects after application onto soil has been widely documented (Sohi et al. 2010; Verheijen et al. 2010). In this study, ANOVA and Tukey analysis showed that the soil amended with biochar coupled with fertilizer addition had significantly lower pH relative to soil treated with biochar alone (Table 1; Fig. 1b) and this may be due to N nitrification after urea and NPK were added. Similar results were reported by Zhu et al. (2014) 46 days after addition of biochar together with N fertilizer to acidic red soils (Oxisols and Ultisols) of southern China with corn as a test crop.

There was no significant polynomial trend detected for the effect of biochar rate on total N concentration of the soil (Table 1). However, polynomial contrast analysis indicated that the existence of biochar rate x fertilizer interaction was mainly due to a significant difference in the linear part of total N responses to biochar rate at different fertilizer rates (Table 1). Application of biochar without fertilizer addition increased total N concentration of the soil, but significant differences over controls ($\text{mean}_{\text{bt}} = 0.17 \text{ g kg}^{-1}$) were only detected at the highest application rate of 15 g kg^{-1} ($\text{mean}_{\text{bt}} = 0.398 \text{ g kg}^{-1}$) (Fig. 2). However, the total N concentration in fertilized soil showed a decreasing trend with the lowest value recorded in soil treated with highest application rate at 15 g kg^{-1} ($\text{mean}_{\text{bt}} = 0.215 \text{ g kg}^{-1}$) of biochar. Reduction of total N with increasing rate of biochar in soil treated with biochar in combination with fertilizer addition was attributed either to: (i) greater N uptake by corn or (ii) utilization and immobilization of N by microorganisms during the decomposition of mineralizable fractions of biochar that have low N contents (Parton

et al. 2007). Burger and Jackson (2003) suggested that greater C availability stimulated microbial activity, resulting in greater N demand, promoting immobilization and recycling of NO_3^- , thus reducing the N availability.

In general, total C concentration (Fig. 3a) in the soil increased significantly with the increasing rate of biochar application ($\text{mean}_{\text{bt}} = 6.54, 8.88, \text{ and } 11.94 \text{ g kg}^{-1}$ for 5, 10 and 15 g kg^{-1} biochar, respectively) (Table 1) and significantly greater compared to control ($\text{mean}_{\text{bt}} = 4.35 \text{ g kg}^{-1}$). This result reflects the increasing amount of organic matter added to the soil with the increasing rate of biochar application. Total C concentration increased as a linear function of biochar rate ($r^2=0.72, p<0.001$) with the biochar increasing the soil total C by 3% for every g kg^{-1} applied. Uzoma et al. (2011) reported that biochar amendments to a dryland sandy soil in Japan significantly increased total C with increasing application rates. Addition of biochar together with mineral fertilizers at the rates of 5 and 15 g kg^{-1} showed a small but non-significant decrease in total C concentration compared to that of the biochar alone (data not shown). As noted by Dil (2011), application of biochar together with nitrogen fertilizer in short-term experiments has been shown to decrease soil C concentration through an effect known as “priming”. This priming effect occurs when previously dormant populations of soil microorganisms are activated by the addition of a readily consumable C or N source, which leads to C mineralization (Kuzyakov et al. 2000). Despite having C with high stability over long periods of time, biochar contains small fractions of C that are easily mineralizable within weeks or months (Kuzyakov et al. 2009; Nguyen et al. 2010). Therefore, when the EFB biochar was applied onto this sandy Podzol together with N fertilizer, the positive effects of biochar on soil carbon may have been negated by the additional N which caused these carbons to mineralize through the priming effect.

Soil CEC increased linearly by $0.09 \text{ cmol kg}^{-1}$ for every g kg^{-1} of biochar added (Fig. 3b). Significant increase in CEC was only detected in the soil treated with 10 and 15 g kg^{-1} of biochar relative to control. Glaser et al. (2003) suggested that oxidation of the aromatic C and formation of carboxyl groups were believed to be the main reason for the observed CEC increase. However, CEC of the treated soil was low ($< 3.5 \text{ cmol kg}^{-1}$) although high amount of biochar was applied. It is possible that the amount of biochar applied was insufficient to increase the CEC beyond this value because the period of the study might not have been sufficient for C oxidation. Duxbury et al. (1989) reported that much of negative charge was not expressed in soils because it was pH dependent and most of the negative charge sites were blocked by interactions with Al. Soil organic matter can effectively increase CEC at pH above 5.5 which is consistent with blockage of exchange sites either by Al or Fe at lower pH values (Steiner et al. 2007). In our study, soil treated with 10 and 15 g kg^{-1} of biochar had pH values higher than 5.5 and hence the CEC was significantly increased.

The availability of K, Ca and Mg in the soil was significantly affected by biochar rate, either applied singly or in combination with inorganic fertilizer. However, only biochar applied at 10 and 15 g kg^{-1} were statistically different compared to the control treatment. Linear polynomial contrast was significant for the response of exchangeable K, Ca and Mg (Table 1). The significant linear regression ($r^2=0.80$, $p<0.001$; $r^2=0.68$, $p<0.001$, for exchangeable K and Mg, respectively) showed that concentrations of exchangeable K and Mg were raised by 10% and 7%, respectively, per g kg^{-1} of biochar applied (Fig. 4a). The exchangeable Ca also increased linearly by 2.85 mg kg^{-1} for every g kg^{-1} of biochar added (Fig. 4b). The biochar used in this study had a higher amount of K than Ca or Mg. Therefore, a much greater increase of soil K was induced by incorporation of the biochar to this sandy soil while there was less impact on the

level of Ca and Mg. This was shown by very low exchangeable Ca ($<80 \text{ mg kg}^{-1}$) and Mg ($<70 \text{ mg kg}^{-1}$) in the soil due to biochar addition. Similar results of K increase were reported by Lehmann et al. (2003a) 37 days after wood biochar was incorporated into an Oxisol in the Brazilian Amazon, Chan et al. (2007) 42 days after adding green waste biochar to an Australian Alfisol and Uzoma et al. (2011) 85 days after addition of cow manure biochar to a dryland sandy soil in Japan. This suggests that biochar addition as amendment could provide a good nutrient source for K in sandy soils (Yuan and Xu 2010).

Effects of biochar application on dry matter production and height of corn

There was a significant biochar rate \times fertilizer interaction on DM production of the above ground biomass of corn (Table 2). There was a significant linear polynomial contrast for the response of DM to biochar rate (Table 2). The fitted linear regression ($r^2=0.64$, $p<0.001$; $r^2=0.50$, $p<0.01$, for unfertilized and fertilized soil, respectively) showed that biochar raised the DM production of corn by 3% for both unfertilized and fertilized soil for every g kg^{-1} added (Fig. 5). Polynomial contrast analysis also showed that the existence of the interaction in the response of DM to biochar rate was mainly due to significant difference in the cubic part of the response function between unfertilized and fertilized soil (Table 2). A significant increase in DM was observed in all biochar treatments ($\text{mean}_{\text{bt}} = 3.01, 2.19, \text{ and } 4.15 \text{ g}$ for 5, 10 and 15 g kg^{-1} of biochar, respectively) relative to the control ($\text{mean}_{\text{bt}} = 0.49 \text{ g}$) for soil with no fertilizer (Fig. 5). A similar result was recorded for DM of corn grown on soil treated with biochar together with inorganic fertilizers, but with greater values ($\text{mean}_{\text{bt}} = 3.13, 4.13, 9.06, 9.06 \text{ g}$ for 0, 5, 10, and 15 g kg^{-1} of biochar, respectively) and the magnitude of DM increased with the rate of biochar

applied. However, significant increase was only observed at 10 and 15 g kg⁻¹ of biochar application. Apparently, combination of 5 g kg⁻¹ biochar and mineral fertilizers in this study did not improve DM production because the application rate was too low and most of nutritional benefits required for growth were likely derived directly via mineral fertilizers application. Uzoma et al. (2011) also found that biochar application at the rate of 15 and 20 t ha⁻¹ plus NPK fertilizer on a sandy soil significantly increased DM of corn relative to NPK-only control, but no difference was found at a lower application rate of 10 t ha⁻¹.

Corn height was significantly affected by biochar rate and fertilizer addition and increased linearly as a function of biochar rate ($r^2=0.36$, $p<0.001$). The height of corn increased by 2.45 cm with every g kg⁻¹ increased in the amount of biochar applied (Fig. 6a). No significant difference was detected at the low rate of 5 g kg⁻¹ of biochar application; significant difference was only detected at higher rate of 10 and 15 g kg⁻¹. Generally, corn grew significantly higher on soil treated with biochar in combination with fertilizer than on soil treated with biochar alone (Fig. 6b).

Effects of biochar application on nutrient concentration and their uptake by corn

A significant biochar rate x fertilizer interactions was detected for N and Mg concentrations in corn tissue (Table 2). Only the linear polynomial contrast was significant for the responses of N to biochar rate (Table 2). The significant linear regression indicated that total N concentration in corn tissue of fertilized soil decreased linearly ($r^2=0.34$, $p<0.05$) by 2.4% with every g kg⁻¹ increased in the amount of biochar applied (Fig. 7a), whereas, there was no significant trend ($r^2=0.16$, $p>0.05$) in the unfertilized soil. The existence of biochar rate x fertilizer interaction was

attributable to the significant difference in the quadratic and cubic components of N concentration response to biochar rate between unfertilized and fertilized soil (Table 2). The fertilized soil seemed to have greater tissue N concentration with values $> 20 \text{ g kg}^{-1}$ compared to the unfertilized soil with the N concentration values $< 15 \text{ g kg}^{-1}$. There was a significant linear polynomial contrast ($r^2=0.51$, $p<0.01$; $r^2=0.36$, $p<0.05$, for unfertilized and fertilized soil, respectively) for the response of Mg concentration to biochar rate (Table 2). The Mg concentrations in corn tissue were raised by 0.05 and 0.07 g kg^{-1} with every g kg^{-1} of biochar applied, for unfertilized and fertilized soil, respectively (Fig. 7b). Polynomial contrast also showed that the existence of the interaction in the response of Mg concentration in corn tissue to biochar rate was mainly due to a significant difference in the quadratic part of the response function between unfertilized and fertilized soil (Table 2). A significant decreased of Mg concentration in tissue of corn grown on unfertilized soil was observed when biochar was applied at 10 g kg^{-1} and 15 g kg^{-1} compared to control. Whereas in fertilized soil, no significant difference of Mg concentration in corn tissue was detected relative to control.

Concentration of Ca in corn tissue was significantly affected by application of biochar and fertilizer addition (Table 2). There was a significant linear response of Ca concentration to biochar rate (Table 2). The significant linear regression ($r^2=0.57$, $p<0.001$) showed that the Ca concentration decreased by 3% for every one g kg^{-1} increased in the amount of biochar applied (Fig. 8a). A significant and negative effect on Ca concentration was observed due to the application of 10 and 15 g kg^{-1} of biochar ($\text{mean}_{\text{bt}} = 1.3$ and 1.15 g kg^{-1} , for 10 and 15 g kg^{-1} , respectively) compared to the control ($\text{mean}_{\text{bt}} = 4.31 \text{ g kg}^{-1}$). Concentrations of Ca in tissue of corn grown on fertilized soil ($\text{mean}_{\text{bt}} = 2.55 \text{ g kg}^{-1}$) were significantly greater than unfertilized soil ($\text{mean}_{\text{bt}} = 1.77 \text{ g kg}^{-1}$) (Fig. 8b). Concentration of K in corn tissue was only affected due to

application of different rates of biochar (Table 2). There was a significant linear polynomial contrast on the responses of K concentration in corn tissue to biochar rate (Table 2). The fitted linear regression ($r^2=0.45$, $p<0.001$) showed that an increase in the amount of biochar added by one g kg^{-1} resulted in an increase of K concentration by 0.67 g kg^{-1} (Fig. 8c). In contrast to N, Mg and Ca concentrations, the tissue K concentration was significantly higher in all soil treated with biochar than the control and the values (except for 0 g kg^{-1} biochar) were above the critical values defined for K of 20 g kg^{-1} (Campbell and Plank 2000). The increase in tissue K concentration as a result of biochar application could be attributed to the high K content in the EFB biochar (69 g kg^{-1}) which was reflected by the higher exchangeable K in the biochar treated soil at harvest. Butnan et al. (2015) also reported increased corn tissue K and decreased Ca and Mg concentration with rates of biochar added to an Ultisols in Thailand.

Result indicates that application of biochar without fertilization resulted in deficiency of N, Mg and Ca in the corn tissue. This was shown by deficient level of N, Mg and Ca concentration in the corn tissue, which was considerably lower than their critical values of 30, 2.5 and 4 g kg^{-1} , respectively (Campbell and Plank 2000). It was observed that the plant tissue N, Ca and Mg concentrations decreased with increasing biochar rates while at the same time DM yields increased. This could be explained by “dilution effect”, where the amount of nutrients present in the plant tissue was sufficiently diluted by the increased DM which consequently reduced the nutrient concentrations (Jarrell and Beverly 1981). In this study, biochar application with or without fertilizer addition positively affected the DM production of the above ground biomass of corn; therefore, inconsistency in nutrient concentrations in the plant tissue may be due to differences in DM production. The dilution effects of yield and DM production on plant

nutrient concentration has been reported previously by Chan et al. (2007) and Rogovska et al. (2014).

The uptake of N and K showed a significant interaction effects between biochar rate and fertilizer addition (Table 2). The N uptake increased as a linear function of biochar rate ($r^2=0.78$, $p<0.001$; $r^2=0.27$, $p<0.05$, for unfertilized and fertilized soil, respectively) with the biochar increasing the N uptake by 0.3% and 0.01% for unfertilized and fertilized soil, respectively, for every g kg^{-1} applied (Fig. 9a). The existence of biochar rate x fertilizer interaction in the response of N uptake to biochar rate was mainly due to a significant difference in the cubic part of the response function between unfertilized and fertilized soil (Table 2). In comparison to the control, application of biochar with no fertilizer increased N uptake with increasing biochar rates and the value remained $< 0.04 \text{ g plant}^{-1}$ but N uptake became much greater and increased to $> 0.1 \text{ g plant}^{-1}$ in the presence of fertilizer. In the presence of inorganic fertilizers, N uptake was decreased due to application of 15 g kg^{-1} biochar. Nitrogen limitation might be the reason for declining DM production at high application rates, given that biochar used in this study had low N content and N availability was reduced through immobilization at high C:N ratios (Lehmann et al. 2003b). This was evident from the low N concentration in tissue at high application rate. Several previous studies also found decreased in N uptake with biochar addition particularly at high rate of application due to biological N immobilization (Gaskin et al. 2008; Rondon et al. 2007, Sika 2012). The results of this study are consistent with the findings of Lehmann et al. (2003a) who reported that N nutrition of cowpea decreased, while biomass production and N uptake increased by biochar additions. There was a significant linear response of K uptake to biochar rate (Table 2). The significant linear regression ($r^2=0.68$, $p<0.001$; $r^2=0.71$, $p<0.001$ for unfertilized and fertilized soil, respectively) showed that the K uptake increased by 0.01 and 0.02

g plant⁻¹ with every one g kg⁻¹ increased in the amount of biochar applied, for unfertilized and fertilized soil, respectively (Fig. 9b). The significant effect of the biochar rate x fertilizer interaction was mainly due to a significant difference in the linear and cubic part of K uptake response to biochar rate between unfertilized and fertilized soil (Table 2). Potassium availability in the soil, its content in the tissue and the uptake increased the most due to biochar application and these likely resulted directly from the presence of considerable amount of K in the biochar itself.

No significant effects of biochar rate were observed in the uptake of Ca and Mg (Table 2). The similar case was also detected for interaction effects between biochar rate and fertilizer addition. However, the uptake of Ca and Mg was only significantly affected if fertilizers were applied. Addition of fertilizer significantly increased uptake of Ca and Mg (mean_{bt} = 0.017 and 0.012 g plant⁻¹, for uptake of Ca and Mg, respectively) than no fertilized treatment (mean_{bt} = 0.004 and 0.002 g plant⁻¹, for uptake of Ca and Mg, respectively) (Fig. 10). This may be attributed to higher DM production of corn in the soil applied with fertilizers compared to that without. There was no significant polynomial trend detected on the effects of biochar rate on Ca and Mg uptake (Table 2). Results suggest that significant increased of exchangeable Ca and Mg in soil by biochar addition do not guarantee the availability of these cations for corn uptake. Therefore, we postulated the low uptake of Ca and Mg were attributable either to; (i) very low content of the Ca and Mg in biochar and slow release of these cations due to strong bonding with the biochar; (ii) antagonistic effect between the uptake of these cations with K uptake. In the latter, the antagonistic effects will occur when there is high uptake of K by corn tissue and would restrict Ca and Mg uptake (Marschner 1995; Butnan et al. 2015). Respective to the initial soil properties, although the cations concentration were very low, the content of Ca was the highest

followed by content of Mg and K. However, application of biochar which contains high amount of K induced a greater exchangeable K increased in the soil compared to exchangeable Ca and Mg and this condition led to the observed of antagonistic effect. This was also shown by deficient levels of Ca and Mg in the corn tissue due to high K consumption.

CONCLUSIONS

This study showed that application of EFB biochar at different rate to sandy Podzol in Malaysia increased the soil pH linearly with concomitant decrease in exchangeable Al. It was observed that application of biochar which contained large amount of C (~50 %) contributed to significant increase of organic matter reserve in this Podzol, leading to linear increased of CEC. Furthermore, decomposition of the EFB biochar would add N, K, Ca and Mg into the otherwise very poor soils, improving its fertility significantly. The uptake of N and K were found to increase linearly with the addition of biochar, whereas no significant polynomial trend was observed for the uptake of Ca and Mg. These positive changes resulted in the significant increase in the DM production and corn height. The data shown proved that application of biochar to the sandy soil had positive and negative impacts on soil and crop growth in a short-term pot trial. Therefore, addition of biochar together with appropriate inorganic and organic fertilizer such compost can be implemented in order to overcome the negative impacts and to maximize soil and crop productivity. Under the conditions of this experiment, the appropriate rate of EFB biochar was found to be between 10 to 15 g kg⁻¹. This rate may be relevant only to horticultural applications that utilize pots for short term responses. However, it is not necessarily applicable to field setting because the impact of biochar on soil properties and crop growth may differ.

Although limited to a short-term pot experiment, this research provide evidence that biochar is a promising material to alleviate the low fertility of sandy soils found in Malaysia. Hence, it is worth to conduct a long-term field experiment to make a comprehensive assessment of biochar application on agronomic and environmental effects.

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Table 1. Probability (*p*) values derived from ANOVA for soil chemical properties 45 days after corn growth under biochar at rates of 0, 5, 10, and 15 g kg⁻¹

Source	pH	TC ^z	TN ^y	CEC ^x	Exchangeable cations			
					K	Ca	Mg	Al
Rate	<0.001	<0.001	0.52	<0.001	<0.001	<0.001	<0.001	<0.001
Fertilizer	0.01	0.67	0.39	0.99	0.29	0.27	0.24	0.32
Rate*Fertilizer	0.17	0.46	0.02	0.38	0.07	0.76	0.22	0.27
Linear	<0.001	<0.001	0.44	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.002	0.65	0.56	0.44	0.36	0.61	0.11	0.89
Cubic	0.61	0.84	0.26	0.22	0.07	0.45	0.15	0.32
Fertilizer*rate linear	0.05	0.70	0.001	0.10	0.10	0.76	0.29	0.53
Fertilizer*rate quadratic	0.44	0.62	0.25	0.78	0.57	0.34	0.69	0.93
Fertilizer*rate cubic	0.49	0.14	0.58	0.68	0.10	0.73	0.08	0.06

^zTotal carbon

^yTotal nitrogen

^xCation exchange capacity

Table 2. Probability (*p*) values derived from ANOVA for above dry matter production, plant height, nutrient concentration and uptake of corn after 45 days of corn growth under biochar at rates of 0, 5, 10, and 15 g kg⁻¹

Source	DM ^z	Corn height	Nutrient concentration in tissue				Nutrient Uptake			
			N	K	Ca	Mg	N	K	Ca	Mg
Rate	<0.001	<0.001	0.001	<0.001	<0.001	0.001	0.001	<0.001	0.74	0.14
Fertilizer	<0.001	<0.001	<0.001	0.33	0.02	<0.001	<0.001	<0.001	<0.001	<0.001
Rate*Fertilizer	0.03	0.12	0.01	0.19	0.16	0.03	0.02	0.002	0.45	0.20
Linear	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.56	0.11
Quadratic	0.11	0.83	0.64	0.08	0.14	0.39	0.06	0.49	0.35	0.11
Cubic	0.42	0.37	0.01	0.32	0.25	0.06	0.06	0.46	0.93	0.57
Fertilizer*rate linear	0.89	0.29	0.64	0.05	0.83	0.44	0.07	0.001	0.31	0.37
Fertilizer*rate quadratic	0.50	0.51	0.02	0.70	0.03	0.01	0.11	0.80	0.48	0.18
Fertilizer*rate cubic	0.001	0.04	0.01	0.39	0.83	0.20	0.03	0.02	0.29	0.14

^zDry matter production of above ground part of corn

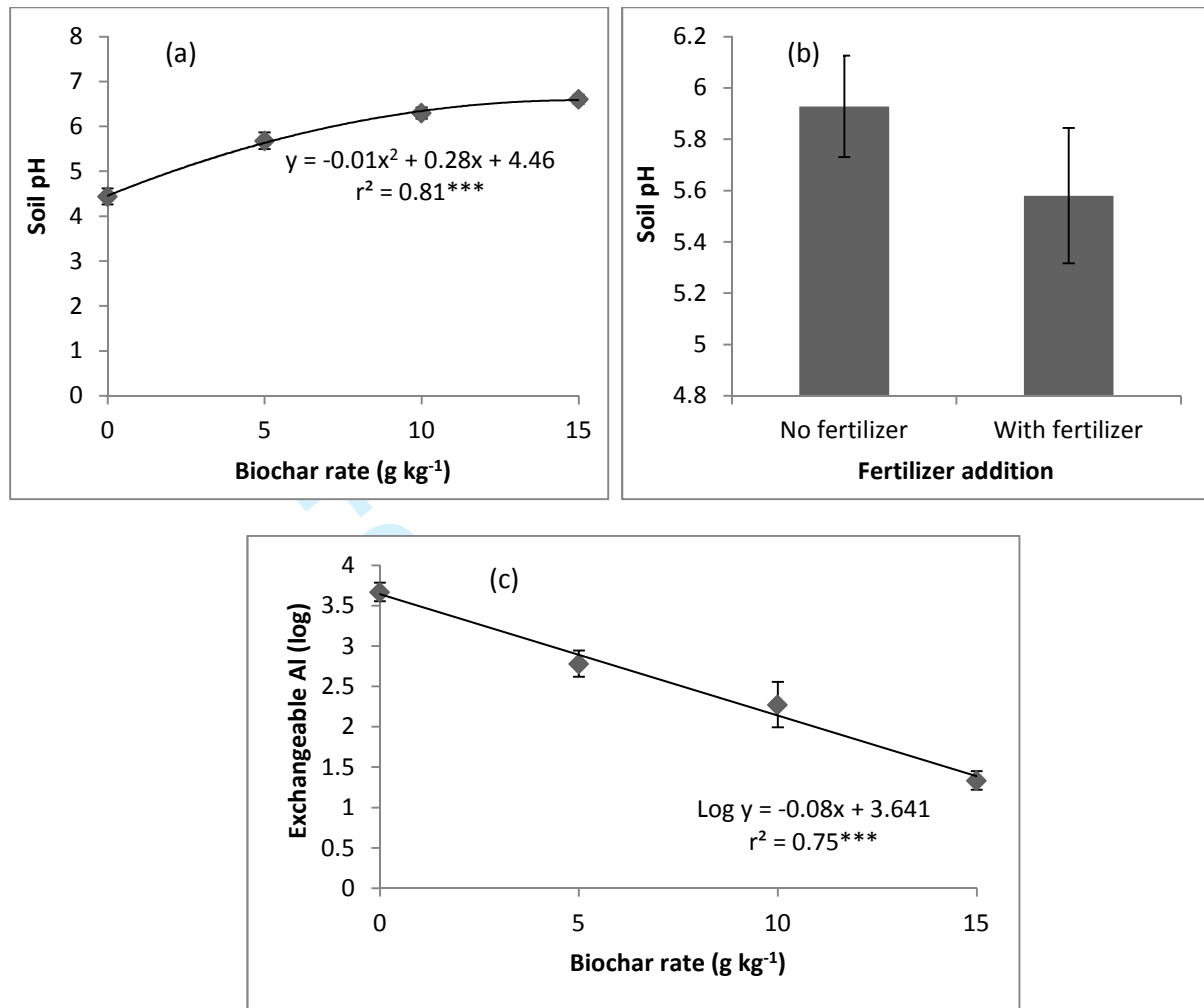


Fig. 1: Soil pH as affected by biochar rate (a) and fertilizer addition (b), and exchangeable Al in log scale (c) as affected by application of different rates of biochar. Error bars represent \pm standard error of the transformed means with $n = 8$ (a and c) and $n = 16$ (b). Significant level: *** $p < 0.001$.

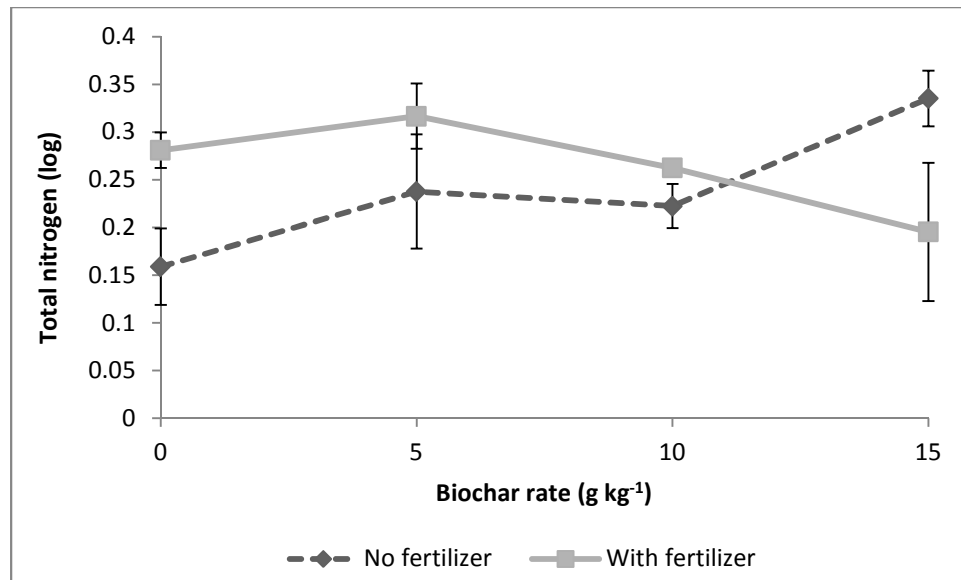


Fig. 2: Interaction effects of biochar rate and fertilizer addition on total N in soil. Error bars represent \pm standard error of the log transformed means ($n = 4$).

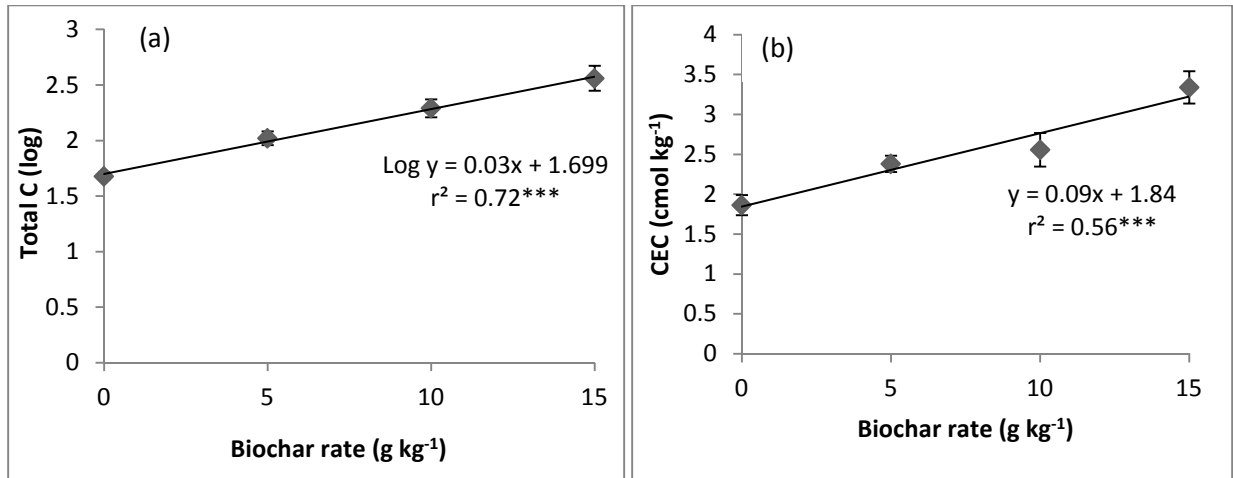


Fig. 3: Soil total C in log scale (a) and CEC (b) as affected by biochar rate. Error bars represent \pm standard error of the log transformed means (a) and the original means (b) with $n = 8$. Significant level: *** $p < 0.001$.

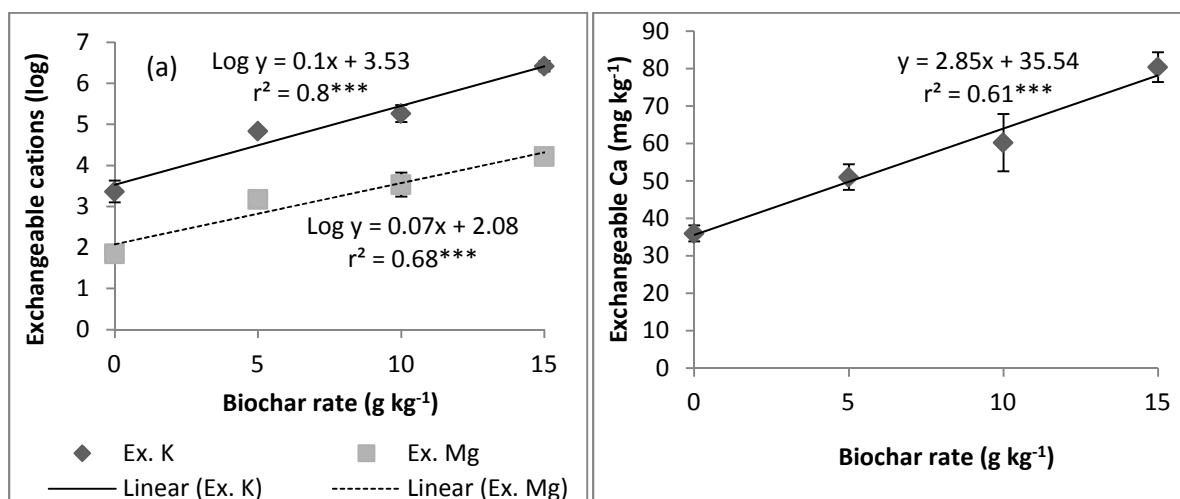


Fig. 4. Soil exchangeable K and Mg in log scale (a) and exchangeable Ca (b) as affected by biochar rate. Error bars represent \pm standard error of the log transformed means (a) and the original means (b) with $n = 8$. Significant level: *** $p < 0.001$.

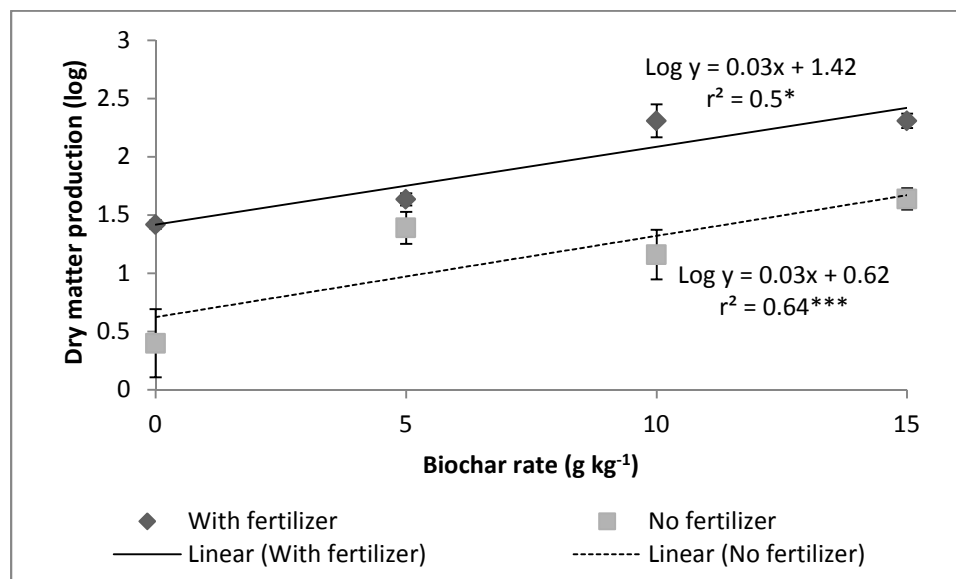


Fig. 5: Interaction effects of biochar rate and fertilizer addition on above ground corn dry matter production. Error bars represent \pm standard error of the log transformed means ($n = 4$). Significant level: ** $p < 0.01$, *** $p < 0.001$.

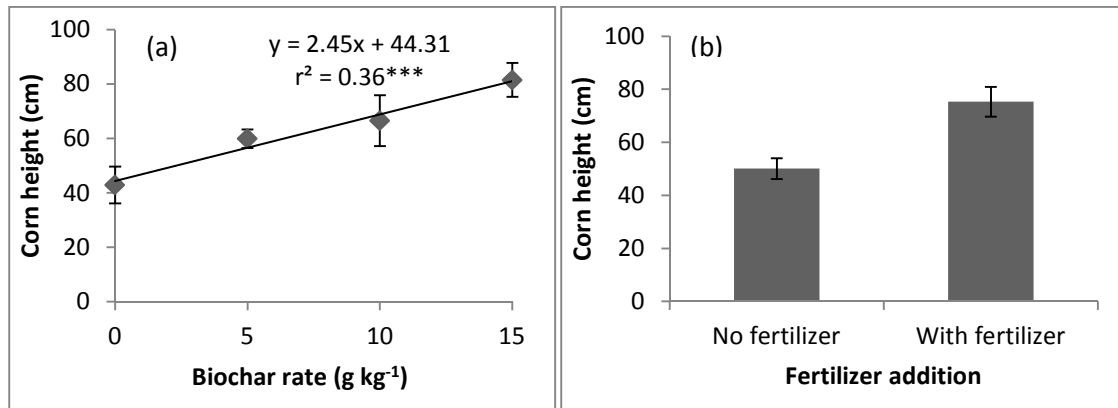


Fig. 6: Corn height as affected by biochar rate (a) and fertilizer addition (b). Error bars represent \pm standard error of the means with $n = 8$ (a) and $n = 16$ (b). Significant level: $*** p < 0.001$.

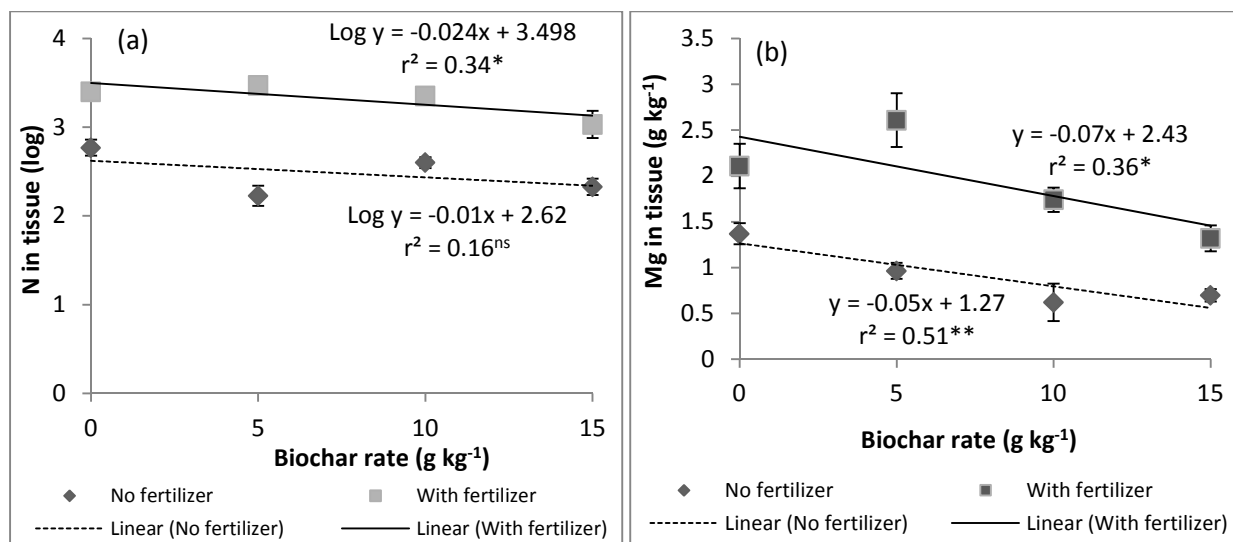


Fig. 7: Interaction effects between biochar rate and fertilizer addition on nitrogen in log scale (a) and Mg concentration (b) in corn tissue. Error bars represent \pm standard error of the log transformed means (a) and original means (b) with $n = 4$. Significant level: ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$.

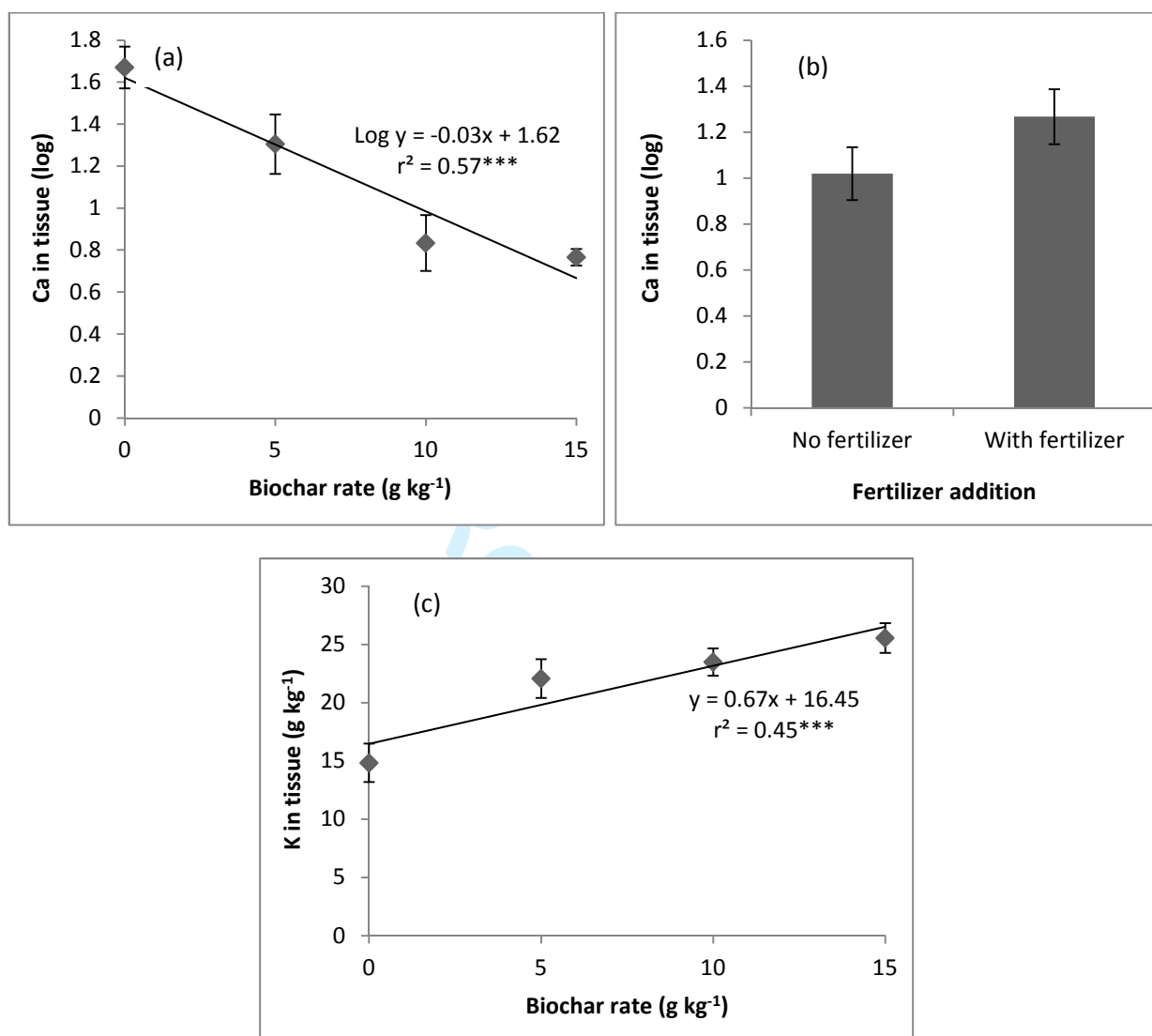


Fig. 8: Log scale of Ca concentration in corn tissue as affected by biochar rate (a) and fertilizer addition (b) and concentration of K in corn tissue as affected by biochar rate (c). Error bars represent \pm standard error of the log transformed means (a and b) and original means (c) with $n = 8$ (a and c) and $n = 16$ (b). Significant level: $*** p < 0.001$.

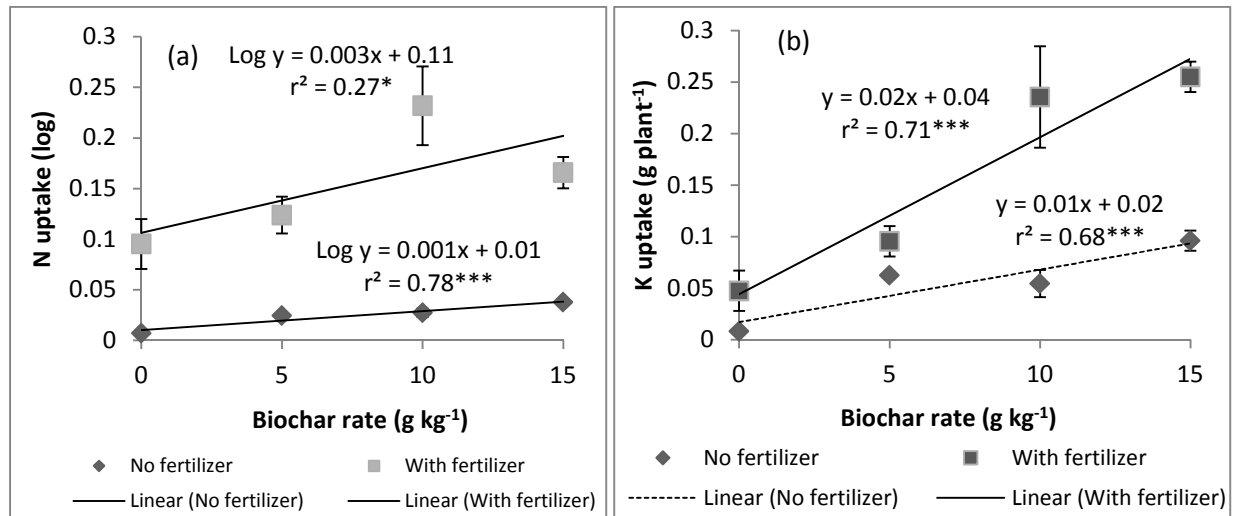


Fig. 9: Interaction effects between biochar rate and fertilizer addition on the uptake of N in log scale (a) and K (b) by corn. Error bars represent \pm standard error of the log transformed means (a) and original means (b) with $n = 4$. Significant level: * $p < 0.05$, *** $p < 0.001$.

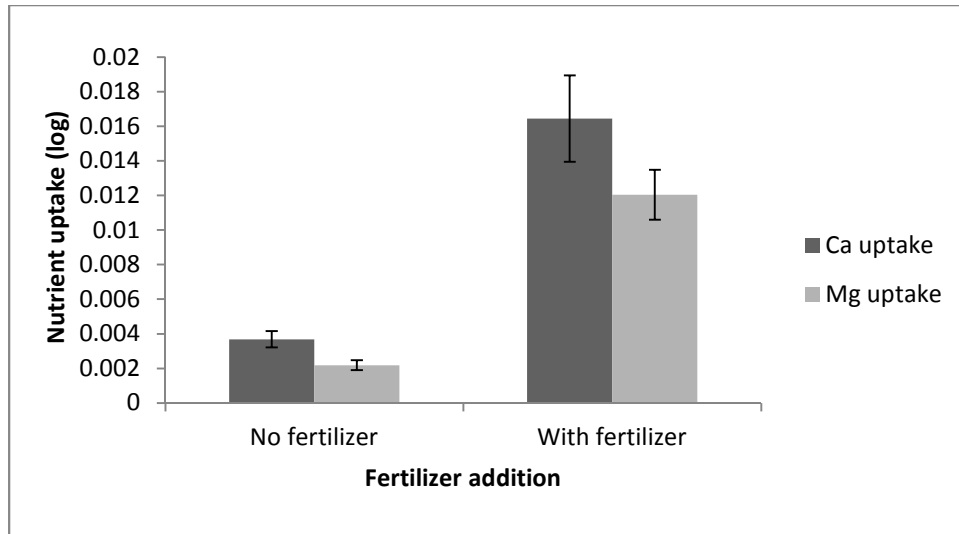


Fig. 10: Uptake of Ca and Mg by corn as affected with fertilizer addition. Error bars represent \pm standard error of the log transformed means ($n = 16$).