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Corn (Zea mays L.) growth, nutrient uptake and soil fertility improvement of strongly acidic soil applied with biochar and animal manure

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ABSTRACT

The effects of biochar and animal manure application on soil chemical properties, nutrient uptake, growth, and biomass production of corn grown in strongly acidic soil were assessed in a pot experiment. The experiment was laid out in randomized complete block design with the following treatments: (1) control; (2) 15 t chicken manure (CM) ha⁻¹; (3) 30 t CM ha⁻¹; (4) 15 t carabao dung (CD) ha⁻¹; (5) 30 t CD ha⁻¹; (6) 15 t chicken manure biochar (CMB) ha⁻¹; (7) 30 t CMB ha⁻¹; (8) 15 t carabao dung biochar (CDB) ha⁻¹; and (9) 30 t CDB ha⁻¹. Application of 30 t CM ha⁻¹ significantly increased soil pH by 1.03-unit, total organic carbon, total N, and exchangeable K by 138%, 300%, and 955%, respectively, and a 108-fold increase in P, over the control treatment. Similarly, the addition of CM at 15 t ha⁻¹ significantly increased all soil chemical parameters gathered. Moreover, the addition of 15 t CM ha⁻¹ increased plant height, shoot, and total biomass by 62%, 161%, and 148% over the control treatment. Meanwhile, tissue N uptake of corn increased by 147% and 124% with the CM application at the rate of 15 t ha⁻¹ and 30 t ha⁻¹. Among organic materials evaluated, CM had the most superior influence on soil chemical properties, growth, biomass production, and plant nutrition.

1. INTRODUCTION

Acid soil is one of the common problem soils often encountered by most upland farmers in the Philippines. Soil becomes acidic when aluminum (Al), iron (Fe), and hydrogen (H) ions dominate the soil exchange sites and soil solutions [1]. The development of acid soil in the country is attributed to natural and human-induced activities. Natural soil acidification through the slow process of soil formation has resulted in the widespread occurrence of acid soil in the country [2]. This acidification process is promoted by high precipitation and high temperature. Human activity such as intensive and continuous cropping without nutrient addition as commonly practiced in the upland areas has also exacerbated the development of acid soil.

In the country, there are about 8.15 million hectares of acidic upland soils which are mostly planted to different crops such as *Ipomoea batatas*, *Manihot esculenta*, Musa acuminata, and other crops [3].

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These areas are characterized with low crop productivity due to low inherent soil fertility, a high concentration of soluble toxic ions such as Al, Fe, and manganese (Mn), low base saturation and cation exchange capacity, low organic matter, and low available phosphorus (P) [3]. A common staple crop grown in these soils is corn (Zea mays), the second most important food crop next to rice in the country. This crop serves as a major source of livelihood for approximately one-third of Filipino farmers [4]. In the Northern and Western part of Mindanao and Visayas region, corn served as a staple food during periods of rice shortage and is also utilized as raw materials for livestock and poultry feeds production. In 2016, corn production registered a 3.99% yield reduction from the previous harvest [5]. Moreover, a significant yield difference across regions in the country was also observed. Soil acidity and declining soil fertility are among the factors that have contributed to the observed yield gap. With the high demand for this crop, it is, therefore, necessary to develop a sound management strategy to increase corn production, especially when grown in unproductive soils.

The use of locally available organic materials such as chicken manure (CM) and carabao dung (CD) is seen as one of the economically viable sources of nutrients in crop production. Animal

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manures usually have an alkaline pH and an excellent source of nitrogen (N), P, potassium (K), and other micronutrients [6–9]. Moreover, manure addition improves soil health by increasing the levels of soil organic matter [10,11]. It also promotes soil granulation and improves soil tilth, thus providing a better rooting medium for plants. Although the addition of raw manure increases soil fertility, enhances plant nutrition, and increases crop yield [12–15], its benefits are short-lived, which usually last only for one or two cropping seasons due to rapid mineralization, especially under hot and wet environment [16]. This rapid mineralization necessitates farmers to apply organic material every year. Thus, the conversion of raw manure into a more stable material such as biochar offers a more long-term benefit.

Biochar refers to organic waste materials either derived from plant or animal heated at high temperature (350°C-650°C) in the absence of oxygen [17]. The resulting charcoal material is known as biochar. Unlike its uncharred counterpart, biochar is more stable and is more resistant to microbial decomposition [18], and thus, providing a long-term benefit to the plants. Biochar application improves soil fertility by neutralizing soil acidity, providing readily mineralizable nutrients, raising soil organic matter, and promoting microbial growth and activity. Additionally, biochar may alter soil physical properties by increasing soil porosity and reducing bulk density, thus, improving water and nutrient retention [19-22]. Many studies have indicated that biochar application to soil could significantly improve soil fertility and increase crop yield [23-27]. Thus, biochar has the potential as an alternative fertilizer, particularly in nutrient-depleted soil.

Biochar addition on degraded soil has gained much attention due to the apparent benefits to soil quality and crop yield. Moreover, biochar's ability to neutralize acid soil and enhance soil fertility depends on the pyrolysis temperature, the type of feedstock, and the application rate. In addition, there is a need to explore more about the potential effects of biochar and animal manure as a potent fertilizer, particularly on strongly acidic soil. Therefore, the main objective of this study was to identify the most promising amendment that can maximize soil fertility, plant growth, and nutrition on strongly acid soil. The specific objectives of this study were to determine the effects of biochar, CM, and CD application at varying rates on (1) soil chemical properties, (2) plant growth, (3) biomass production, and (4) N and P uptake of corn grown in strongly acidic soil.

2. MATERIALS AND METHODS

2.1. Soil Collection, Preparation and Analyses

The acid soil used in the study was collected from Brgy. Taganahaw, Butuan City. Soil samples from 0 to 20 cm depth were randomly collected in the area. The collected samples were air-dried for three days in the screen house, pulverized using a wooden mallet and sifted using a 4-mm mesh screen. Before potting, a 1 kg soil sample was set aside for pH, total organic carbon (OC), N, P, K, calcium (Ca), magnesium (Mg), sodium (Na), and Fe analysis. The analysis was carried out at the Regional Soils Laboratory of the Department of Agriculture, Brgy. Taguibo,

Butuan City. Meanwhile, the remaining soil samples were used for the pot experiment.

2.2. Biochar Production, Manure Preparation and Chemical Characterization

Poultry manure and CD were sourced out from a local farm at Brgy. Taguibo, Butuan City. The two manures were air-dried separately for five days and sifted to 2 mm. Biochar was produced using a fabricated pyrolyzer [19]. Biochar production was done by heating the biomass (manure) at 400°C–500°C for 4–6 hours under anaerobic conditions, a process known as pyrolysis. The biochar produced was allowed to cool overnight and was later sifted using a 2-mm mesh. After cooling of biochar, a thorough chemical characterization (pH, total OC, total N, P, K, Ca, Mg, and Na) followed. Meanwhile, the remaining biochar was set aside for potting preparation. Similarly, the nutrient content of manures was also measured.

2.3. Pot Preparation and Bagging

A total of 36 polyethylene bags measuring 20 cm diameter and 35 cm in height were used in this study. Each bag was filled with 6 kg of non-sterilized soil on an oven-dry weight basis. The sieved animal manure and biochar were thoroughly mixed in the soil and incubated for 20 days. Every five days, the soil-biochar mixture was mixed thoroughly until planting. Similarly, the CM and CD amended pots were mixed thoroughly and incubated for 20 days. All treatments (control, animal manure, and biochar-amended soils) were added with tap water up to 70% moisture content of field capacity.

2.4. Experimental Design

There were 36 treatment combinations established in a randomized complete block design. The treatments are as follows: $T_1 = \text{control}$, $T_2 = 15 \text{ t CM ha}^{-1}$, $T_3 = 30 \text{ t CM ha}^{-1}$, $T_4 = 15 \text{ t CD ha}^{-1}$, $T_5 = 30 \text{ t CD ha}^{-1}$, $T_6 = 15 \text{ t chicken manure biochar (CMB) ha}^{-1}$, $T_7 = 30 \text{ t CMB ha}^{-1}$, $T_8 = 15 \text{ t carabao dung biochar (CDB) ha}^{-1}$, and $T_9 = 30 \text{ t CDB ha}^{-1}$. Each treatment was four times replicated. The pot experiment was set up inside the greenhouse of the College of Agriculture and Agri-Industries, Caraga State University, Butuan City, Philippines.

2.5. Pot Experiment

Five seeds of corn [National Seed Industry Council (NSIC) Cn 08-222 variety] were sown in each of 36 polyethylene bags. The seeds were allowed to grow for 15 days and later thinned to one plant per pot. The plants were regularly watered with tap water, and the moisture content was kept at 70% of field capacity. Weeds were removed manually after emergence, whereas insects were removed by handpicking. Plants were harvested at 70 days after transplanting. Harvesting was done by cutting the base of the plants and by carefully removing the roots from the soil. Any soils adhering to the roots were removed by washing. The shoots and roots were washed with tap water three times. The plant samples were finally rinsed with distilled water and air-dried for three days after blot drying. The different plant parts were oven-dried using a forced draft oven set at 70°C for

three days. After oven drying, the dried shoots and roots were weighed. The oven-dried tissue samples were submitted to the Regional Soils Laboratory for N and P analysis. On the other hand, soil samples from each of the 36 pots were air-dried for three days. After air-drying, samples were sieved (2 mm) and 200 g subsamples from each bag were collected and submitted to the laboratory for chemical analyses.

2.6. Statistical Analysis

Analyses of variance were performed using Statistical Tool for Agricultural Research (STAR) version 2.0.1 2014 to determine the significance of the treatments. Post hoc analysis using Tukey's Honest Significant Difference Test was done to compare each treatment means.

3. RESULTS AND DISCUSSION

3.1. Initial Chemical Properties of Soil

Table 1 presents the initial chemical analysis of the soil used in the study. Soil pH was strongly acidic with a very low total OC. The N, P, K, Ca, Mg, and Na contents were also deficient. On the other hand, the total Fe concentration in the soil was extremely high. The overall poor soil fertility status leads to poor crop performance. Deficiency symptoms such as chlorosis, stunted growth, and necrosis were also visible in the control plants.

3.2. Chemical Properties of CM, CD, and Biochar

Table 2 presents the analytical results of the different soil organic amendments used in the experiment. The analysis showed that CM had higher N, P, K, and Ca content compared with CD, except for Mg and Na. Pyrolytic reaction (heating the feedstock at high temperature under limited air condition) increased the pH of the biochar (CMB and CDB) compared to its uncharred counterpart (CM and CD). The order of pH increase is as follows: CMB>CDB>CM>CD. All organic materials tested were highly alkaline, except CD. The pH of the feedstock increased with pyrolysis similar to the observation by Conz et al. [18]. Moreover, pyrolysis also increased the P, K, Ca, Mg, and Na content in the materials. The biomass feedstock greatly influenced the nutrient composition and properties of biochar. In contrast, biochar production had decreased the total OC of CM and CD by 47% and

Table 1: Chemical characteristics of soil.

Property	Soil
pH (1:5 soil to H ₂ O)	4.80
OC (%)	0.23
Total N (%)	0.07
Extractable P (mg kg ⁻¹)	4.00
Exchangeable bases (mg kg ⁻¹)	
K	72.00
Ca	BDL
Mg	0.26
Na	47.50
Total Fe (mg kg ⁻¹)	29,460.74

^{*}BDL = below the detection limit

42%, respectively. The reduction of total OC in both biochars was due to the conversion of carbon (C) into CO_2 during the burning process, particularly at a higher temperature and severe pyrolysis condition. Similarly, total N also decreased with pyrolysis by 49% in CMB and 6% in CDB due to NH₃ volatilization.

3.3. Effects of CM, CD, and Biochar Application on Soil Chemical Properties After Harvest

Table 3 presents the influence of biochar and animal manure on soil chemical properties after harvest. All soil chemical parameters examined significantly differed with the addition of organic amendments. Application of the CMB and CM at 30 t ha⁻¹ significantly increased soil pH by 1.09 and 1.03 units, respectively, over the control treatment. Likewise, the addition of 15 t ha⁻¹ CMB increased the pH by 0.31 unit. On the contrary, CD, CDB, and control treatments have similar effects on soil pH. There was even a slight reduction in pH when CD and CDB were applied. Higher pH recorded in soil amended with CMB compared to CDB is in agreement with the chemical analysis of the two materials. As shown in Table 2, CMB is more alkaline than CDB. Therefore, it is reasonable that the soil treated with CMB had a high pH. The CM biochar application increased the soil pH similar to the observation reported by Furtado et al. [28]. The pH increase was due to the alkaline nature of biochar. Also, Mandal et al. [29]

Table 2: Chemical characteristics of the different organic amendments used in the study.

Property	CM	CD	CMB	CDB
рН	8.82	7.65	10.97	9.35
Total Organic Carbon (TOC) (%)	22.31	23.79	11.87	13.89
Total (%)				
N	2.62	1.46	1.34	1.38
P	8.24	1.27	9.12	2.17
K	4.30	0.66	6.55	0.74
Ca	7.46	BDL	11.05	10.31
Mg	1.11	1.41	1.69	1.74
Na	26.25	482.50	55.25	650.00

*BDL = below the detection limit

Table 3: Means for the residual effects of CM, CD, and biochar application on selected soil chemical properties.

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Treatment	pН	TOC	Total N	Extractable P	Exchangeable K
Treatment pri		(%)		(mg kg ⁻¹)	
Control	4.76 c	0.21 d	0.02 d	1.33 g	72.00 d
15 t ha ⁻¹ CM	4.63 c	0.37 b	0.05 b	71.00 c	387.33 c
$30 \text{ t ha}^{-1} \text{ CM}$	5.79 a	0.50 a	0.08 a	143.00 a	759.33 a
15 t ha ⁻¹ CD	4.61 c	0.25 cd	0.03 cd	3.33 fg	81.00 d
$30 \text{ t ha}^{-1} \text{ CD}$	4.64 c	0.33 bc	0.04 c	6.67 f	132.33 d
$15 \text{ t ha}^{-1} \text{ CMB}$	5.07 b	0.25 cd	0.03 cd	34.67 d	327.67 c
$30 \text{ t ha}^{-1} \text{ CMB}$	5.85 a	0.29 bcd	0.03 cd	76.33 b	563.00 b
15 t ha ⁻¹ CDB	4.67 c	0.23 d	0.02 cd	4.67 fg	89.00 d
$30 \text{ t ha}^{-1} \text{ CDB}$	4.74 c	0.29 bcd	0.04 c	12.33 e	116.00 d
<i>p</i> -value (<0.05)	**	**	**	**	**

Means in a column followed by common letters are not significantly different at 5% level of significance; ns = not significant; ** = significant at <0.01

found a significant increase in soil pH with the CM application. They attributed this increase to the initial high pH and high basic cation content of the material.

Soil total OC after harvest ranged from 0.21% to 0.50%. The addition of 30 t CM ha⁻¹ resulted in the highest total OC. The recorded increase was 138% higher over the control treatment. A marked improvement on soil total OC was also recorded on soils treated with 15 t CM ha⁻¹ and 30 t CD ha⁻¹. In contrast, biochar application did not increase soil total OC levels. As pointed out in Table 2, both biochars have lower OC content. The substantial increase in soil total OC with CM and CD at 30 t ha⁻¹ was due to the high C content of these two materials. This observation is in agreement with those obtained by Onwu et al. [30] and Moyin-Jesu [10] who had reported an increase in soil OC levels with the CM application.

Apart from enriching soil OC, the application of CM improved other soil properties too such as total N. The highest total N value was recorded in the 30 t CM ha⁻¹ amended soil. The N content was increased by 300% over the untreated pot. This N enrichment with CM application was due to the high availability of N in the material (Table 2). Similarly, the addition of 15 t CM ha⁻¹, 30 t CD ha⁻¹, and 30 t CDB ha⁻¹ also increased total N by 150%, 100%, and 100%, respectively, over the control treatment. However, at the same application rate (30 t ha⁻¹), it was clear that CM was two times more effective in increasing soil total N than CD and CDB. Opara et al. [31] and Eneje et al. [32] also reported the same significant increase in soil N concentration with the CM application at increasing rates.

Biochar and animal manure application significantly increased soil P levels after harvest. Application of 30 t CM ha⁻¹ registered a 108-fold increase over the control treatment. Consequently, the addition of 30 t CMB ha⁻¹, 15 t CM ha⁻¹, 15 t CMB ha⁻¹, 30 t CDB ha⁻¹, and 30 t CD ha⁻¹ at decreasing order markedly increased soil P over the untreated pot. In contrast, soil P did not differ significantly between 15 t CDB ha⁻¹, 15 t CD ha⁻¹, and the control treatments. Direct P addition from biochar and animal manure and increased P retention in soils might have contributed to the overall increase in soil P. Also, higher P availability in CM and CMB amended pots reflect the initially high P content from these materials. Mahmood et al. [33] also found an increase in soil available P with the application of 13 t CM ha⁻¹. Similarly, Sonmez et al. [34] found higher P availability in animal manure amended soils.

Consistently, the addition of CM positively influenced soil K at harvest. Pots amended with 15 t CM ha⁻¹ increased soil K by 438% over the control treatment. At 30 t ha⁻¹ application rate, soil K further increased by 955%. Similarly, CMB application at increasing rates significantly increased soil K. Application of CMB at the rate of 15 t ha⁻¹, and 30 t ha⁻¹ increased soil K by 355% and 682%, respectively, over the control treatment. Although the addition of both CM and CMB increased soil K, it was clear that the effect of CM was more superior to CMB. According to Khan et al. [35], CM contains a large amount of potentially mineralizable nutrients. Thus, adding to the soil provides more available nutrients to the plants. In contrast, CD, CDB, and control treatments have a similar and less effect on soil K. The effects of CD on soil K are not surprising since the

material had lower inherent K than CM (Table 2). The increase in soil exchangeable K following application of CM and CMB showed that a large amount of K was introduced from these materials. Islam et al. [36] also reported these positive effects of CM application on soil chemical properties.

3.4. Effects of CM, CD, and Biochar Application on Plant Height and Biomass of Corn

Table 4 shows the different plant measurements recorded at harvest. The parameters include plant height, root, shoot, and total dry matter. Plant height at harvest as influenced by different organic fertilizer treatments significantly differed at a 5% level. Application of 15 t CM ha⁻¹ and 30 t CM ha⁻¹ significantly increased plant height over the control plants by 62% and 87%, respectively. This finding concurs with the result of Enujeke [37] who reported the highest corn plant height in plots amended with 30 t CM ha⁻¹. Higher nutrient availability, soil pH improvement, and better plant nutrition in the CM treatment have resulted in superior growth. Meanwhile, plants in the control treatment were observed to have a very inferior growth compared to those applied with organic fertilizers. On the other hand, CMB, CD, and CDB applications did not show a significant influence of plant height.

Similarly, the addition of CM at the rate of 15 t ha⁻¹ and 30 t ha⁻¹ significantly increased plant biomass production. Shoot dry weight in plants amended with 15 t CM ha⁻¹ significantly increased by 161% over untreated plants. Also, the addition of 30 t CM ha⁻¹ increased shoot dry weight by 128%. Plant total dry weight follows a similar trend with shoot dry weight. The dry weight values ranged from 6.93 g pot⁻¹ to 20.92 g pot⁻¹. Heavier dry weights were recorded in the 15 t CM ha⁻¹ and 30 t CM ha⁻¹ treatments. At 15 t CM ha⁻¹ application rate, the plant was 148% heavier over the control. When the application rate was raised to 30 t CM ha⁻¹, the weight increase was 108%. In contrast, plant dry weights in CD, CDB, CMB, and the control treatments were comparable. The order of increase in total dry weights was 15 t CM ha⁻¹ >30 t CM ha⁻¹ >15 t CMB ha⁻¹ >30 t CD ha⁻¹ >30 t CDB ha⁻¹.

Table 4: Means for the effects of CM, CD, and biochar application on plant height, root, shoot, and total biomass of corn.

neight, root, shoot, and total promises of com-					
Treatment	Height at harvest (cm)	Dry weight (g plant ⁻¹)			
Treatment		Root	Shoot	Total	
Control	78.42 c	1.15 ab	7.31 c	8.45 c	
$15 \text{ t ha}^{-1} \text{ CM}$	127.25 ab	1.82 a	19.10 a	20.92 a	
$30 \text{ t ha}^{-1} \text{ CM}$	146.75 a	0.98 ab	16.63 ab	17.61 ab	
15 t ha ⁻¹ CD	92.75 bc	0.92 ab	8.21 c	9.13 c	
$30 \text{ t ha}^{-1} \text{ CD}$	105.75 abc	1.41 ab	10.65 bc	12.05 bc	
15 t ha ⁻¹ CMB	117.50 abc	1.04 ab	11.71 abc	12.75 abc	
$30 \text{ t ha}^{-1} \text{ CMB}$	116.58 abc	0.39 b	10.51 bc	10.90 bc	
15 t ha ⁻¹ CDB	90.17 bc	0.60 b	6.33 c	6.93 c	
$30 \text{ t ha}^{-1} \text{ CDB}$	90.33 bc	0.59 b	10.77 bc	11.36 bc	
<i>p</i> -value (<0.05)	**	**	**	**	

Means in a column followed by common letters are not significantly different at 5% level of significance; ns = not significant, ** = significant at <0.01.

The present findings reveal that CM application even at the lower application rate on strongly acidic soil can enhance corn growth and biomass production. Moreover, improvement in soil fertility and plant nutrition also resulted in better crop performance. Our results corroborate with the findings of Uwah et al. [38] who reported an increase in corn dry matter production with CM application at the rate of 15 t ha⁻¹. Similarly, Agbede et al. [39] reported the same increase in plant height and biomass production with CM applications in acidic soil. Likewise, substantial improvement in corn biomass with CM application was also reported by Kareem et al. [40].

3.5. Effects of CM, CD, and Biochar Application on Tissue N and P Concentration and Uptake of Corn

Tissue N and P concentration and uptake of corn as influenced by organic fertilizer application are presented in Table 5. Tissue N uptake significantly increased with CM application, regardless of the rate. N uptake of corn in pots applied with 15 t CM ha⁻¹ was higher by 147% over the control treatment. When the CM application rate was doubled, the increase was 124%. Meanwhile, both the CMB and control treatments had comparable N uptake values.

Similarly, the N content of plants in CD treatment did not differ with CDB. The increase in N nutrition of corn grown in CM amended pots suggests that N in CM was more available from this material. As pointed out in Table 2, it was clear that the total N content in CM was higher than that of CMB, CD, and CDB. Moreover, the application of CM increased soil N content. Thus, it is reasonable that CM amended plants absorb more N than the control treatment. The findings of the present study agree with that of Hirzel et al. [41], who reported a significant increase in N uptake of corn for two years of cropping following CM application. Waniyo et al. [42] also reported higher N uptake with CM application at $10 \text{ t ha}^{-1} - 30 \text{ t ha}^{-1}$.

Biochar and animal manure application did not enhance tissue P concentration and P uptake (Table 5). However, plants amended with CM, CMB, and CDB absorbed more P than the control plants. Although the P content in the soil temporarily increased with

Table 5: Means for the effects of CM, CD, and biochar application on total N and P concentration and uptake of corn.

T	Concentr	ration (%)	Uptake (mg	Uptake (mg plant ⁻¹)	
Treatment	N	P	N	P	
Control	1.94	0.17	162.20 c	14.30	
15 t ha ⁻¹ CM	1.92	0.17	400.96 a	35.06	
30 t ha ⁻¹ CM	2.03	0.28	363.17 ab	47.55	
15 t ha ⁻¹ CD	1.48	0.14	133.74 с	14.65	
30 t ha ⁻¹ CD	1.72	0.14	208.72 bc	17.33	
15 t ha ⁻¹ CMB	1.81	0.38	222.98 abc	42.28	
30 t ha ⁻¹ CMB	2.08	0.29	226.88 abc	31.61	
15 t ha ⁻¹ CDB	2.05	0.32	137.85 с	22.23	
30 t ha ⁻¹ CDB	2.03	0.73	232.04 abc	109.09	
<i>p</i> -value (<0.05)	ns	ns	**	ns	

Means in a column followed by common letters are not significantly different at 5% level of significance; ns = not significant; ** = significant at <0.01.

manure and biochar application, the said increase was not sufficient to raise the P content in the plant at a significant level. Moreover, the Fe content in the soil was extremely high, which probably limits P availability to the plants (Table 1). Fe binds and precipitates soil P rendering it unavailable for plant absorption [43].

4. CONCLUSION

The study demonstrates the potential use of biochar and animal manure as valuable amendments for improving soil fertility and corn growth on strongly acidic soil. Among organic materials evaluated, CM at 15 t ha⁻¹ and 30 t ha⁻¹ had the most superior influence on soil chemical properties, growth, biomass production, and plant nutrient concentration. However, follow up studies under field conditions should be conducted to validate the results of the investigation.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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