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Article

Okra Yield Increased by Bat Guano beyond Its Nutrient Supply

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Abstract: The difficulty in obtaining commercial fertilizers by smallholder farmers in sub-Saharan Africa makes it very important to optimize the use of local organic resources. In VilanKulo, Mozambique, a study was carried out on okra (*Abelmoschus esculentus*) over two growing seasons in a haplic Lixisol, a loamy-sand textured soil, applying both bat guano and biochar prepared by an artisanal process. Bat guano was applied at two rates (5 and 10 t ha^{-1}), one month and just before sowing. Biochar was also used at two rates (5 and 10 t ha^{-1}) applied close to sowing. Mixtures of biochar and guano at two rates (1 and 4 t ha^{-1}, and 2 and 8 t ha^{-1}, respectively) were also used along with a non-fertilized control. Field trials were arranged in a completely randomized design with three replicates. The treatments receiving high rates of guano tended to show significantly higher fruit yields (> 10 t ha^{-1}, being the two-year average) in comparison to the control, which showed the lowest average okra fruit yield (6.21 t ha^{-1}). In guano treatments, the apparent recovery by okra of some important nutrients, such as nitrogen (N), was greater than the amount of nutrient contained in the guano itself. This result, together with many others related to tissue nutrient concentration, soil properties and residual fertilizing value in guano plots, indicates a strong mineralization of guano during the growing season, probably due to its low carbon (C)/N ratio and favourable environmental conditions for the mineralization process, such as high temperature and well-aerated soil. The result also suggests some kind of manuring effect, i.e., a fertilizing effect of guano beyond what can be explained by the nutrient supply. The use of biochar increased total organic C in the soil and cation exchange capacity (CEC) compared to the control, but did not affect variables related to plant performance, such as tissue nutrient concentration or plant growth and yield.

Keywords: abelmoschus esculentus; tropical savanna climate; biochar; soil amendment; manuring effect; nutrient mining

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1. Introduction

The world production of okra [*Abelmoschus esculentus* (L.) Moench] has increased over recent decades, reaching 10,822,249 t in 2021 [1]. Currently, the main producing continent is Asia (7,124,510 t), followed by Africa (3,600,881 t), the Americas (81,698 t) and Europe (9,146 t) [1]. Okra can be grown in tropical, subtropical and warm temperate climates. Given this, and because it can be cultivated not only for food but also for several industrial purposes [2], it has the potential to become an even more economically important crop.

Okra is commonly valued for its green immature edible fruits. They contain water (~ 90%), carbohydrates (~ 7%), protein (~ 2%), fibres (alpha-cellulose, hemicellulose, lignin, pectin, …), some important water-soluble vitamins and minerals such as calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K) and zinc (Zn) [3]. Several okra organs, including fruits, leaves, roots, seeds and flowers, are harvested for ethnobotanical uses [4]. It has also been proved that okra may have many medicinal properties, such as antidiabetic, diuretic, anticancer, antioxidant, ophthalmic, cardiac and neurological effects [5,6,7]. The possibility that okra consumption might also have beneficial effects against SARS-CoV-2 has also been reported [8]. Furthermore, due to the high content and quality of oil in its seeds, and wide ecological adaptation, okra can be considered a contender as a new source of non-edible oil for biodiesel production on bioenergy farms [9]. Okra has enormous potential to improve livelihoods in urban and rural areas of sub-Saharan countries, due to the advantage of it being grown during the long warm season in these regions [10]. In contrast, most vegetables providing minerals and vitamins are not well adapted to warm and dry climates due to their sensitivity to harsh environmental conditions [11].

Another important issue for growing vegetables in Africa is the low fertility of the soil [12,13]. Hence, a sustainable increase in okra production and simultaneous improvement in livelihoods of small-scale farmers in the tropics can only be achieved by appropriate soil fertility management techniques. In the tropics, soil nutrients have been mined by small-scale farmers for decades because they do not use sufficient amounts of manure or fertilizer. A sustainable approach to soil fertility management requires replenishing nutrients that are taken from the soil by cultivated plants [14,15]. There are different nutrient replacement techniques, but the most common is the use of commercial fertilizers, to which small-scale farmers do not generally have access, for socioeconomic and geographic reasons. Thus, farmers must use all available fertilizing materials, especially organic amendments, to maintain soil fertility and the productivity of their crops.

In Mapinhane, Vilankulo district, southern Mozambique, there are natural deposits of bat excrement, named guano, that present a great opportunity for farmers to fertilize their crops. Previous studies have shown a positive effect of applying guano in the improvement of soil properties and crop yields [16,17,18]. Farmers in this region have also learned how to make biochar through artisanal processes. Several studies have also shown that biochar may enhance relevant soil properties, thereby improving its fertility [19,20,21]. Biochar may also improve crop growth and yield. A study using biochar in okra, carried out under drought stress conditions, showed a significant increase in plant growth (plant height by 14.2% and root dry weight by 30.0% over the control) and in several root morphological traits (projected area by 22.3% and root diameter by 22.7% over the control) [20]. The use of biochar has also been tested in combination with NPK fertilizers, with results showing an improvement in various soil properties [21]) or crop yield [22].

In this study, the effect of applying bat guano at rates of 5 and 10 t ha^{-1} both one month before and at the time of okra sowing was assessed. The field experiments also included the application of biochar at rates of 5 and 10 t ha^{-1} at sowing, and two mixtures of guano and biochar, 1 and 4 t ha^{-1} and 2 and 8 t ha^{-1}, respectively, together with a control treatment. The hypotheses established for this study were: i) soil properties and/or crop productivity were improved by the application of organic amendments; ii) the early application of guano improves its effect on plants by bringing forward the release of nutrients; and iii) the mixture of guano and biochar has synergistic effects that improve crop productivity.

2. Materials and Methods

2.1. Experimental conditions

A two-year field trial was carried out in VilanKulo district, Joint Aid Management Life (JAM - Life) farm (21° 59' 05'' S, 35° 09' 39'' E) in Pambara Administrative Post, southern Mozambique. The plot where this trial took place has been cultivated with maize (*Zea mays* L.) in monoculture for five years. VilanKulo experiences a semi-arid climate. Under the Köppen classification, it is located in Aw type, which corresponds to a tropical savanna climate, or wet and dry climate [23]. Two main seasons can be identified in Vilankulo: the hot and rainy season, which runs from October to March; and the dry and cool season, which runs from April to September [24]. Rainfall is erratic and is the environmental variable that raises major problems for the cultivation processes. The long-term average annual precipitation and temperature are 677 mm and 24.2 °C, respectively. The monthly variation is shown in Table 1 [25].

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Table 1. Average monthly temperature and precipitation in VilanKulo (1991 - 2021).

Month	Temperature (°C)	Precipitation (mm)
January	26.9	142
February	26.9	151
March	26.3	85
April	24.6	40
May	22.7	20
June	21.3	14
July	20.6	13
August	21.6	8
September	23.1	11
October	24.3	23
November	25.6	67
December	26.6	102

The soil is a haplic Lixisol, according to the FAO classification [26], very weathered and derived from limestone [27]. Some soil properties of the plots where the study was carried out, determined from samples taken at 0-0.20 m, are shown in Table 2.

Table 2. Selected soil properties (average±standard deviation, n=3) from composite samples (10 cores taken per composite sample) taken at 0-0.20 m depth at the beginning of the study.

Soil properties	2018	2019
[1]Organic carbon (g kg^{-1})	4.2±0.24	11.1±1.64
[2]pH (H_2O)	6.6±0.15	6.8±0.18
[3]Extract. P (mg P_2O_5 kg^{-1})	41.8±8.56	75.1±17.94
[3]Extract. K (mg K_2O kg^{-1})	87.2±10.07	90.4±17.57
[4]Exchang. Ca ($cmol_c$ kg^{-1})	3.1±0.19	5.4±0.56
[4]Exchang. Mg ($cmol_c$ kg^{-1})	1.0±0.15	1.4±0.13
[4]Exchang. K ($cmol_c$ kg^{-1})	0.3±0.03	0.2±0.04
[4]Exchang. Na ($cmol_c$ kg^{-1})	0.6±0.13	0.7±0.11
[5]Exchang. acidity ($cmol_c$ kg^{-1})	0.1±0.06	0.2±0.06
[6]CEC ($cmol_c$ kg^{-1})	5.1±0.33	7.9±0.53
[7]Sand	89.5±0.87	84.6±1.03
[7]Silt	2.2±0.51	6.3±0.91
[7]Clay	8.2±0.76	9.2±0.86
[7]Texture	Loamy-sand	Loamy-sand

[1]Wet digestion (Walkley-Black); [2]Potentiometry; [3]Ammonium lactate; [4]Ammonium acetate; [5]Potassium chloride; [6]Cation Exchange Capacity; 7 Robinson pipette method.

2.2. Trial layout and treatments

The field trial was performed during the growing seasons of 2017/2018 and 2018/2019. The experiment was arranged as a factorial design with two factors, year and soil amendment. The soil amendment treatments were: application of 5 t ha^{-1} of guano at sowing time (G5); 10 t ha^{-1} of guano at sowing (G10); 5 t ha^{-1} of biochar at sowing (B5); 10 t ha^{-1} of biochar at sowing (B10); 5 t ha^{-1} of guano one month before sowing [G5(-1)]; 10 t ha^{-1} of guano one month before sowing [G10(-1)]; 1 and 4 t ha^{-1} of biochar and guano, respectively, at sowing (B1G4); 2 and 8 t ha^{-1} of biochar and guano, respectively, at sowing (B2G8); and an unfertilized control (C). All treatments were laid out in the experimental area in three replicates and arranged in plots measuring 3.85 m × 2.40 m.

The guano used in this study was bat excrement, locally available from natural deposits in Mapinhane Administrative Post. This organic material is commonly used by farmers as a soil amendment. Biochar is also a local material prepared from forest residues, collected in sawmills, and spontaneous vegetation. Biochar was prepared through an artisanal method of slow pyrolysis which consists of the heating for one day of biomass (feedstock) in a reactor made from two metallic drums. For more details about slow pyrolysis the reader is referred to Chun et al. [28]. Some properties of the guano and biochar used in this study are shown in Table 3.

Table 3. Selected properties (average±standard deviation, n=3) of guano and biochar used in the experiment.

	Guano		Biochar	
Properties	2018	2019	2018	2019
Moisture (%)	9.1±1.50	8.0±1.73	35.5±3.70	33.9±2.71
[1]Organic carbon (g kg^{-1})	59.8±2.47	57.5±2.87	534.5±14.12	538.2±16.53
[2]pH (H_2O)	7.5±0.17	7.3±0.20	9.2±0.24	9.3±0.20
[3]Nitrogen (g kg^{-1})	3.3±0.40	4.2±0.47	3.3±0.28	5.0±0.35
[4]Phosphorus (g kg^{-1})	10.1±1.65	8.4±1.01	0.8±0.10	0.9±0.09
[4]Boron (mg kg^{-1})	13.7±2.55	15.5±3.59	28.5±2.70	34.6±3.92
[5]Potassium (g kg^{-1})	2.9±0.20	3.9±0.67	3.6±0.52	4.0±0.59
[6]Calcium (g kg^{-1})	0.7±0.08	0.5±0.06	4.3±0.68	4.8±0.34
[6]Magnesium (g kg^{-1})	0.9±0.06	1.1±0.15	1.6±0.17	1.9±0.24
[6]Iron (mg kg^{-1})	28188.0±2720.97	45606.2±4732.90	3637.3±539.37	5679.6±316.57
[6]Manganese (mg kg^{-1})	168.2±17.59	286.3±71.07	364.1±34.16	388.5±43.65
[6]Zinc (mg kg^{-1})	109.7±33.04	112.6±19.19	27.2±5.06	42.1±8.39
[6]Copper (mg kg^{-1})	72.8±14.29	113.3±13.07	72.2±27.81	23.6±4.60

[1]Incineration; [2]Potentiometry; [3]Kjeldahl; [4]Colorimetry; [5]Flame emission spectrometry; [6]Atomic absorption spectrophotometry.

2.3. Field plot management

The plots where this study took place were previously used for the production of maize monoculture. The soil was prepared mechanically by disc plough and disc harrow at the beginning of the hot season in October. Organic amendments were applied on two dates depending on the treatment, one month before and just before sowing.

In the growing season of 2017/2018, guano was applied on November 11 and December 11, 2017, the latter also being the date of application of biochar and sowing. In the following season of 2018/2019, guano was applied on February 8 and March 8, 2019. On March 8, biochar was also applied and sowing was carried out. An open-pollinated variety of okra (cv. Clemson Spineless) was sown at 0.77 m × 0.30 m spacing under a drip irrigation system with an allocation of ~ 2,500 m^3 water per growing season. The control of weeds was performed by hand during the two growing seasons. Okra was harvested twice a year on March 14 and 30, 2018, and the yield reported as the sum of the two harvests. In 2019, the harvests were done on June 23 and July 13.

2.4. Soil and plant sampling and field measurements

Three composite soil samples (10 individual cores per sample) were collected at 0-0.20 m depth for the initial soil characterization. At the end of each annual field trial, the soil was sampled again (5 individual cores per sample), at the same depth for determination of the effect of the treatments on general soil properties and also to carry out a pot experiment to obtain a biological index of soil nutrient availability. After sampling, the soil was air-dried and sieved (2 mm mesh).

Plant height and phenological stages [BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) scale] [29] were assessed periodically during the growing season. Plant height was measured by a meter from the ground to the highest point of the plant (leaves or male inflorescence).

Leaf samples were collected for elemental analysis and the monitoring of plants' nutritional status between the two principal growth stages "6 - Flowering" and "7 - Development of fruit" [29], when the crop had more than fifteen extended internodes. Twelve randomly selected young mature leaves were collected per experimental unit.

Okra was harvested by hand when the fruits were still tender and edible, at 71 growth stage [29], to obtain crop yield. The aboveground biomass of the plant was also collected, weighed and dried to determine the total dry matter yield (DMY) and for determination of nutrient removal and apparent nutrient recovery after determination of tissue elemental composition.

2.5. Pot experiment

Subsamples of the soil taken at the end of the field trial were used for assessing the residual effect of the treatments on cabbage (*Brassica oleracea* L., cv. Tronchuda), grown in a pot experiment. The goal was to obtain a biological index of soil nutrient availability. The pots were filled with 2.5 kg of soil and placed in an open space but with the sides of the pots protected from direct solar radiation with newsprint to prevent overheating of the rooting zone. In the first growing season, the cabbage was grown from July 1 to August 16, 2018, and in the second growing season from August 29 to October 15, 2019. Weeds that germinated in the pots were immediately removed by hand. The plants were watered by applying 150 ml of water per pot whenever there was insufficient precipitation. At phenological stage 18, corresponding to eight or more true unfolded leaves [29], the plants were cut at ground level, oven dried at 70 °C and weighed. Thereafter, the plants were ground (1 mm mesh) for elemental analysis.

2.6. Laboratory analyses

Soil samples were analysed for: 1) pH (H_2O, KCl) (soil: solution, 1:2.5); 2) CEC (ammonium acetate, pH 7.0) and exchange acidity (KCl extraction); 3) easily oxidizable C (wet digestion, Walkley-Black method); 4) total organic C (incineration); 5) extractable P and K (ammonium lactate); 6) extractable boron (B) (hot water extraction and azomethine-H methods); 7) extractable Fe, Zn, manganese (Mn) and copper (Cu) (ammonium acetate and EDTA, determined by atomic absorption spectrometry). In the initial samples there were also determined 8) soil separates (clay, silt and sand fractions) (Robinson pipette method). Methods 1–5, and 8 are fully described by [30], method 6 by [31] and method 7 by [32].

Tissue samples (okra fruits, leaves and stalks, and cabbage tissues), and organic amendments, were oven-dried at 70 ºC until constant weight, ground (1 mm mesh) and analysed for elemental composition. Elemental tissue analyses were performed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K) and atomic absorption spectrophotometry (Ca, Mg, Cu, Fe, Zn and Mn) methods, after nitric digestion of the samples [33]. Guano and biochar were also analysed for total organic C by incineration and for pH (organic amendment: solution, 1:2.5).

2.7. Data analysis

The statistical software SPSS Statistics (v. 25, IBM SPSS Chicago, IL) was used for data analysis. Data was firstly tested for normality and homogeneity of variances using the Shapiro-Wilk test and

Levene's test, respectively. The comparison of the effect of the treatments was provided by two-way ANOVA. When significant differences between soil treatments were found (P < 0.05), the means were separated by the multiple range Tukey HSD test (α = 0.05).

3. Results

3.1. Okra fruit yield and plant height

No significant interaction (P = 0.0512) was found between years and soil amendment treatments in okra fruit yield (Figure 1). Crop production, however, varied significantly between years and between soil amendment treatments. Okra fruit yield was particularly higher in 2018 (9.82 kg ha^{-1}) in comparison to 2019 (7.14 kg ha^{-1}). The treatments receiving high rates of guano tended to show higher fruit yields, with the highest average value recorded in the treatment B2G8 (12.16 t ha^{-1}), followed by G10(-1) (10.55 t ha^{-1}) and G10 (10.03 t ha^{-1}). Control treatments displayed the lowest okra fruit yield (6.21 t ha^{-1}), although this value was only significantly lower than those of treatments B2G8 and G10(-1).

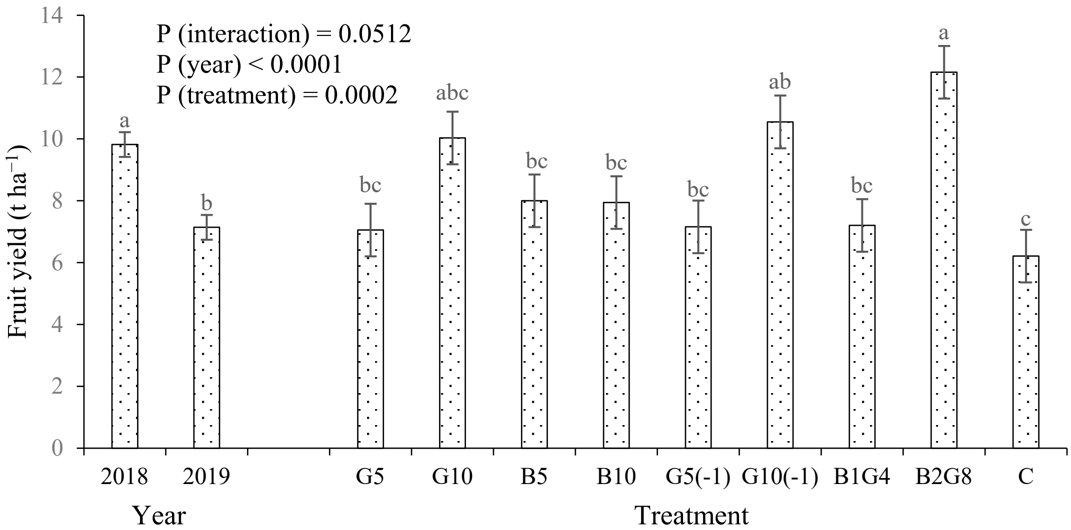

Figure 1. Okra fruit yield as a function of year and soil amendment treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing]. Separately by year and treatments, mean values followed by the same letters are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars indicate the standard errors (n = 3).

The pattern of response to year and soil amendments for the plant height variable (Figure 2) was similar to that reported for okra fruit yield (Figure 1). However, the interaction between the two factors, year and soil amendment, was significant. In 2018, the plants reached a higher height than in 2019 (0.64 and 0.44 m, respectively). As observed for fruit production, treatments receiving high rates of guano gave taller plants, regardless of year, despite the significant interaction between factors. The three treatments in which the plants reached the highest average heights were B2G8 (0.64 m), G10 (0.62 m) and G10(-1) (0.61 m), and the three treatments in which the plants were shorter were G5 (0.46 m), C (0.47 m) and G5(-1) (0.48 m).

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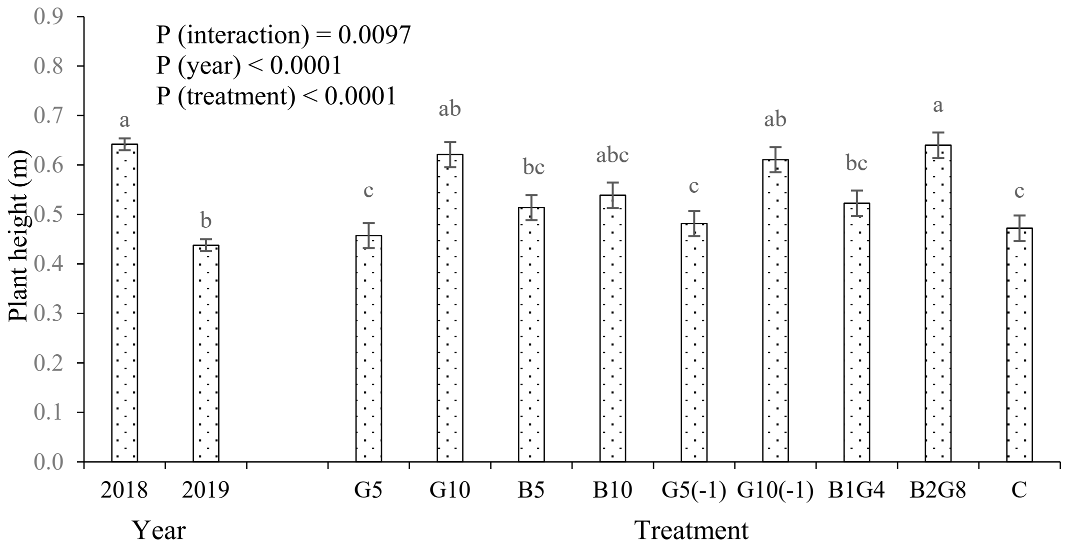

Figure 2. Okra plant height as a function of year and soil amendment treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing]. Separately by year and treatments, mean values followed by the same letters are not significantly different by Tukey HSD test (α = 0.05). Vertical bars indicate the standard errors (n = 3).

3.2. Plant nutritional status and nutrient recovery

Leaf N concentration did not vary significantly with soil amendment treatments in any of the years (Table 4). In 2018, the average values varied between 19.6 and 21.6 g kg^{-1}, and were lower than those observed in 2019, which varied from 28.6 to 31.2 g kg^{-1}, probably as a result of a dilution effect, since in 2018 the plants produced more fruit. In 2018, leaf average N concentrations were below the lower limit of the sufficiency range. Leaf P concentration did not vary significantly between treatments in 2018, whereas in 2019, significant differences were found. A tendency towards higher values was observed in the treatments receiving the higher rates of guano. Thus, the higher average values were found in the treatments G10(-1) (4.7 g kg^{-1}) and G10 (4.3 g kg^{-1}), and the lower values in the control treatment (2.3 g kg^{-1}). Leaf K concentration did not vary significantly nor was any trend observed between the soil amendment treatments. Leaf B levels showed significant differences between treatments and a clear trend to higher values in the treatments receiving the higher rates of guano, the pattern being observable over the two years of study. The other analysed macro and micronutrients behaved identically to that of K without significant differences and/or any tendency as a function of soil amendment type and rate (data not shown).

Table 4. Leaf nitrogen (N), phosphorus (P), potassium (K) and boron (B) concentration as a function of soil amendment treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing] and lower and higher limits of sufficiency range (LLSR and HLSR, respectively).

	Leaf N (g kg^{-1})		Leaf P (g kg^{-1})		Leaf K (g kg^{-1})		Leaf B (mg kg^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019
G5	21.4a	30.8a	4.1a	3.0cd	16.9a	16.9a	58.4abc	66.5ab
G10	21.5a	31.2a	4.2a	4.3ab	16.3a	22.0a	62.0ab	73.0ab
B5	19.6a	28.6a	3.9a	2.9cd	14.3a	19.3a	57.0abc	52.9b
B10	20.7a	30.2a	3.8a	2.2d	14.4a	17.7a	55.2abc	53.0b
G5(-1)	20.9a	30.3a	4.3a	3.8abc	14.4a	21.6a	57.9abc	69.5ab

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G10(-1)	21.6a	31.2a	4.4a	4.7a	15.3a	19.9a	61.0ab	80.3a
B1G4	20.4a	30.2a	3.8a	2.9cd	19.0a	22.0a	50.7bc	67.7ab
B2G8	21.1a	31.0a	4.1a	3.4bcd	15.9a	19.7a	64.3a	65.8ab
C	20.0a	29.2a	3.9a	2.3d	16.3a	20.6a	46.0c	58.5ab
LLSR	25		3		17		20	
HLSR	45		6		30		50	
Prob > P	0.9502	0.6467	0.7363	<0.0001	0.9621	0.3938	0.0044	0.0216
Std. error	1.25	1.04	0.255	0.27	2.82	1.70	2.72	5.16

In columns, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

Nutrient recovery is estimated from the plant's total DMY and the concentration of nutrients in its tissues. The results obtained for N, P, K and B are presented in Table 5. In comparison with the concentration of nutrients in leaves, which is subject to dilution and concentration effects, this variable showed much clearer differences between treatments. N recovery varied significantly between treatments in 2018 and 2019, and it was clear that the plants grown under the treatments receiving the higher rates of guano displayed the higher average values. Thus, comparing these results with those in Table 4, it seems that they reflect much more the effect of treatments on crop production than on N concentration in plant tissues. P, K and B recovery followed the pattern reported for N. K is the most paradigmatic example, since there was no trend attributable to the effect of the treatments on the K concentration in the leaves, but they showed higher K recovery values associated with the treatments that led to higher crop yields.

Table 5. Nitrogen (N), phosphorus (P), potassium (K) and boron (B) recovery as a function of soil amendment treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing].

	N recovery (kg ha^{-1})		P recovery (kg ha^{-1})		K recovery (kg ha^{-1})		B recovery (g ha^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019
G5	143.1ab	135.5b	29.9bc	17.7bc	151.0ab	177.2c	276.9ab	208.0bc
G10	154.8a	165.8a	37.9abc	32.2a	189.4a	227.9a	350.3ab	291.0a
B5	134.0ab	136.0b	30.1bc	18.8bc	162.4ab	180.4bc	267.5ab	207.7bc
B10	128.1ab	130.6b	32.2bc	13.6c	155.6ab	138.1d	301.6ab	156.9c
G5(-1)	127.4ab	133.0b	34.0abc	18.1bc	140.2ab	166.1c	313.6ab	202.9bc
G10(-1)	147.7ab	170.0a	43.6ab	31.6a	203.4a	229.1a	390.2a	268.3a
B1G4	134.0ab	133.4b	28.1bc	18.0bc	158.6ab	172.2c	268.8ab	194.5bc
B2G8	151.7a	166.1a	50.3a	22.6b	208.5a	204.8ab	382.2a	251.2ab
C	121.1b	126.8b	20.8c	13.8c	111.6b	162.3cd	192.1b	171.2c
Prob > P	<0.0001	<0.0001	0.0006	<0.0001	0.0046	<0.0001	0.0087	<0.0001
Std. error	5.82	5.16	3.47	2.32	32.18	15.81	32.18	11.63

In columns, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

Apparent nutrient recovery measures the amount of nutrient taken up by the plant in relation to the amount of nutrient applied as a fertilizer or a soil amendment. The apparent recovery values of N, P, K and B (ANR, APR, AKR and ABR, respectively) of the treatments that received guano are shown in Table 6. In 2018, ANR ranged from 42.0% [G5(-1)] to 146.8% (G5) and in 2019 from 32.1%

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[G5(-1)] to 127.0% (B2G8). Thus, it seems that the result did not depend on the rate of guano applied. In both years, some values were greater than 100%, which means that the plants took up more N than was contained in the applied guano. APR varied between 18.6 and 40.2% in 2018 and between 10.2 and 23.9% in 2019, also regardless of the guano rate. In 2018, AKR showed particularly high values, well above 100%, and in 2019, the least productive year for okra, values varied between 21.2% and 185.9%. In 2019, it appears that the most productive plots had higher AKR values. ABR ranged from 127.1 to 195.0% in 2018 and between 40.9 and 84.0% in 2019. The values were particularly higher in 2018, the most productive year, compared to 2019. In 2019 the higher values were associated with the most productive plots.

Table 6. Apparent nitrogen, phosphorus, potassium and boron recovery (ANR, APR, AKR and ABR, respectively) as a function of soil amendment treatments [G, guano; B, biochar; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing].

	ANR (%)		APR (%)		AKR (%)		ABR (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
G5	146.8	45.2	19.9	10.2	298.4	83.1	136.2	51.7
G10	112.2	101.0	18.6	23.9	294.4	182.7	127.1	84.0
B5	---	---	---	---	---	---	---	---
B10	---	---	---	---	---	---	---	---
G5(-1)	42.0	32.1	28.7	11.1	216.1	21.2	195.0	44.5
G10(-1)	88.4	111.9	24.8	23.1	347.5	185.9	159.2	68.1
B1G4	107.0	42.8	20.0	13.6	445.1	69.1	153.9	40.9
B2G8	127.3	127.0	40.2	14.3	459.3	147.9	190.9	70.2
C	---	---	---	---	---	---	---	---

Apparent nutrient recovery (%) = 100 × [(Nutrient recovered in the amended treatments – Nutrient recovered in the control treatment)/Nutrient applied as an organic amendment].

3.3. Soil properties

Total organic C varied significantly with treatments in both years (Table 7). B10 recorded the highest average values in 2018 (10.4 g kg^{-1}) and in 2019 (16.9 g kg^{-1}). The control showed low average values in both years, 8.6 and 8.9 g kg-1, respectively, in 2018 and 2019. Easily oxidizable C did not vary significantly between treatments in 2018. In 2019 significant differences were found between treatments, and the higher average values were associated with the treatments receiving high rates of guano, such as G10 (12.4 g kg^{-1}), G10(-1) (12.1 g kg^{-1}) and B2G8 (12.0 g kg^{-1}). Soil pH did not vary significantly with soil amendment treatments, with mean values ranging between 6.6 and 6.9 and 6.8 and 7.0 in 2018 and 2019, respectively. The levels of available P in the soil varied significantly between treatments in the two years. In 2018, the highest values, significantly different from the control, were associated with treatments consisting of the highest biochar rates (B2G8 and G10). In 2019, treatments consisting of high guano rates [G10(-1) and G10] gave the highest values. The control treatment again presented the lowest average value.

Table 7. Total organic carbon (TOC, incineration), easily oxidizable carbon (EOC, Walkley Black), pH $_{(H2O)}$ and extractable phosphorus (P) as a function of fertilization treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing].

	TOC (g kg^{-1})		EOC (g kg^{-1})		pH $_{(H2O)}$		P (mg kg^{-1}, P_2O_5)	
	2018	2019	2018	2019	2018	2019	2018	2019
G5	9.0ab	12.0bcd	3.8a	9.2bcd	6.8a	6.9a	32.8ab	59.1abc
G10	9.4ab	13.8abc	4.0a	12.4a	6.8a	7.0a	37.8ab	89.1ab

B5	9.3ab	15.0abc	3.9a	8.7d	6.8a	6.9a	28.6ab	60.0abc
B10	10.4a	16.9a	4.0a	11.4ab	6.9a	6.8a	40.9a	52.2bc
G5(-1)	8.8ab	11.6cd	3.9a	11.0abc	6.7a	6.9a	37.1ab	74.8abc
G10(-1)	8.8ab	12.7bcd	3.9a	12.1a	6.8a	6.8a	38.5ab	95.0a
B1G4	8.5b	12.3bcd	3.8a	9.0cd	6.8a	6.9a	32.2ab	44.5c
B2G8	10.0ab	16.0abc	3.9a	12.0a	6.8a	6.9a	42.9a	63.7abc
C	8.6b	8.9d	3.8a	8.9cd	6.6a	6.9a	22.0b	41.9c
Prob > P	0.0214	<0.0001	0.8831	<0.0001	0.3921	0.9985	0.0268	0.0008
Std. error	0.35	0.82	0.15	0.47	0.10	0.14	3.77	7.57

In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

Exchangeable Ca varied significantly between treatments in 2019 but not in 2018 (Table 8). In 2019, the highest mean values of exchangeable Ca were found in the treatments that received biochar, namely B10 (7.07 $cmol_c\ kg^{-1}$) and B5 (5.87 $cmol_c\ kg^{-1}$), whose values were significantly different from those of the control (4.16 $cmol_c\ kg^{-1}$). Exchangeable Mg and K did not differ significantly between treatments in any of the years. CEC varied significantly between treatments in 2019 but not in 2018, reflecting the effect of exchangeable Ca on CEC. In 2018, mean values varied between 4.29 (C) and 5.58 (B10) $cmol_c\ kg^{-1}$, and in 2019 between 5.48 $cmol_c\ kg^{-1}$ (G5) and 8.96 $cmol_c\ kg^{-1}$ (B10).

Table 8. Exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+) and cation exchange capacity (CEC) as a function of fertilization treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing].

	Ca^{2+} ($cmol_c\ kg^{-1}$)		Mg^{2+} ($cmol_c\ kg^{-1}$)		K^+ ($cmol_c\ kg^{-1}$)		CEC ($cmol_c\ kg^{-1}$)	
	2018	2019	2018	2019	2018	2019	2018	2019
G5	3.15a	3.88d	0.90a	0.92a	0.36a	0.25a	5.03a	5.48e
G10	3.22a	5.09bcd	0.83a	1.16a	0.36a	0.28a	4.98a	7.26bc
B5	3.19a	5.87ab	0.79a	0.95a	0.27a	0.30a	4.83a	7.55b
B10	3.66a	7.06a	0.97a	1.01a	0.30a	0.34a	5.58a	8.96a
G5(-1)	3.07a	4.22cd	0.80a	1.02a	0.33a	0.25a	4.69a	6.03de
G10(-1)	3.38a	4.69bcd	0.92a	1.16a	0.26a	0.28a	5.14a	6.70cd
B1G4	3.43a	5.26bc	0.81a	1.07a	0.27a	0.30a	5.08a	7.18bc
B2G8	3.66a	5.39bc	0.95a	1.05a	0.27a	0.31a	5.49a	7.31bc
C	2.70a	4.16cd	0.73a	1.00a	0.36a	0.28a	4.29a	5.96de
Prob > P	0.1051	<0.0001	0.1458	0.2236	0.6214	0.0846	0.4728	<0.0001
Std. error	0.21	0.29	0.07	0.07	0.06	0.02	0.33	0.28

In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

3.4. Growth and nutrient uptake by cabbage grown in pots as a biological index of soil nutrient availability

No significant interaction ($P = 0.1872$) was found between years and soil amendment treatments on cabbage DMY (Figure 3). In 2019, cabbage DMY (4.28 g plant^{-1}) was significantly higher than in

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the previous year (3.13 g plant⁻¹). DMY did not vary significantly with the soil amendment treatments and the two-year average values varied between 3.27 and 3.91 g plant⁻¹. Thus, the response pattern of okra in the field to soil amendment treatments was not maintained in the potted cabbage.

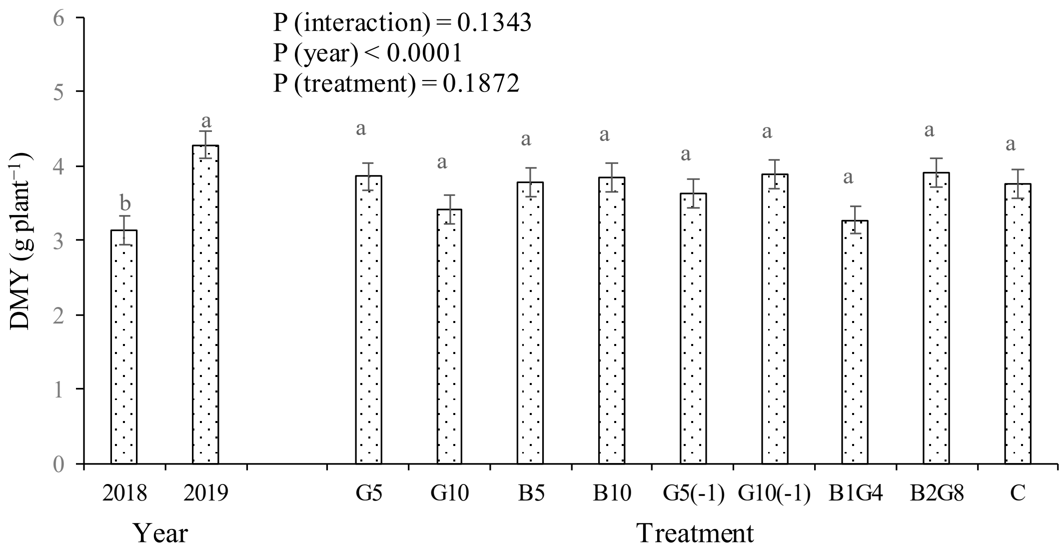

Figure 3. Cabbage dry matter yield (DMY) in the pot experiment as a function of soil amendment treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing]. Separately by year and treatments, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05). Vertical bars indicate the standard errors (n = 3).

In 2018, N concentration in cabbage tissues did not vary significantly between treatments (Table 9). Mean values were found between 14.4 and 16.4 g kg^{-1}. In 2019, significant differences were observed between treatments, with the highest mean value recorded in the G10 treatment (13.4 g kg^{-1}) and the lowest in the control treatment (8.4 g kg^{-1}). Tissue P concentration varied significantly between treatments in both years of the study. In 2018, the highest and lowest mean values were recorded respectively in G10 (3.7 g kg^{-1}) and C (2.9 g kg^{-1}). In 2019, C maintained low mean values, the highest being observed in the B2G8 treatment (3.2 g kg^{-1}) and in general they were also high in the treatments that received high rates of guano. Tissue K concentrations did not vary significantly between treatments. In 2018 the average values varied between 25.4 and 31.0 g kg^{-1} and in 2019 between 16.4 and 21.9 g kg^{-1}. Mean concentration of B in tissues ranged between 27.8 and 35.5 mg kg^{-1} in 2018, with no significant differences between treatments. In 2019, significant differences were observed between treatments, with the highest values recorded in treatment G10 (43.4 mg kg^{-1}) and the lowest in treatment B5 (25.9 mg kg^{-1}). There was a general trend towards higher mean values in the guano treatments.

Table 9. Nitrogen (N), phosphorus (P), potassium (K) and boron (B) concentration in cabbage tissues as a function of soil amendment treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing].

	Tissue N (g kg^{-1})		Tissue P (g kg^{-1})		Tissue K (g kg^{-1})		Tissue B (mg kg^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019
G5	15.4a	11.5ab	3.5ab	1.9ab	26.6a	21.9a	33.4a	39.0ab
G10	16.4a	13.4a	3.7a	2.7ab	28.1a	17.6a	35.5a	43.4a
B5	14.5a	9.7bc	3.0ab	1.9ab	26.9a	16.4a	30.0a	25.9b
B10	16.0a	10.9abc	3.2ab	1.7b	30.0a	18.4a	30.7a	31.9ab

G5(-1)	16.2a	10.5abc	3.4ab	2.6ab	26.7a	17.1a	34.8a	35.8ab
G10(-1)	14.4a	11.3abc	3.5ab	2.4ab	25.4a	19.3a	34.9a	36.1ab
B1G4	16.2a	10.7abc	3.2ab	1.8b	31.0a	19.8a	27.8a	36.0ab
B2G8	15.3a	12.1ab	3.4ab	3.2a	28.6a	17.9a	32.4a	37.1ab
C	16.3a	8.4c	2.9b	1.8b	29.6a	17.6a	27.8a	29.8ab
Prob > P	0.8996	0.0017	0.0463	0.0128	0.1967	0.7672	0.1947	0.0179
Std. error	1.16	0.62	0.18	0.28	1.55	2.15	2.88	2.87

In columns, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

4. Discussion

4.1. Guano increased crop growth and yield, but biochar did not

Okra fruit yield and plant height varied significantly between treatments and years. The treatments that received the highest rates of guano [G10, G10(-1), B2G8] tended to enhance the performance of the crop. Organic amendments generally increase crop productivity as they provide nutrients for plant uptake and improve general plant growth conditions by increasing soil water holding capacity, aeration and many other physical, chemical and biological soil properties [34,35]. Also, in previous studies where bat guano had been used, positive effects on soil properties and/or on variables related to plant growth and yield have been reported [16,17,18,36]. In contrast, treatments receiving only biochar as a soil amendment did not increase plant height or fruit yield compared to the control in these two short-term experiments. Biochar is a C-rich material, difficult to be attacked by microorganisms, with high recalcitrance in the soil, making its use often recommended mainly based on its ability to sequester C [37,38,39]. Even though it contains some essential nutrients, its bioavailability tends to be reduced due to the slow mineralization process, which explains why biochar is mainly seen as a soil conditioner. Biochar can benefit long-term plant growth by improving soil properties [40,41]. However, in short-term assessments, the use of biochar alone, without any other fertilizing materials, often results in low yields, usually no different from control treatments [36,42,43,44].

4.2. Okra plant took up higher amounts of nutrients than those released by guano

Leaf N concentration did not vary significantly between treatments. However, as mentioned above, the treatments with higher rates of guano increased okra growth and yield. N recovery in aboveground biomass also varied significantly between treatments, with the highest values found in the treatments that received the highest rates of guano. Thus, guano increased crop yield and N recovery but not leaf N concentration. The increase in total plant biomass reduced leaf N concentration due to a dilution effect, a phenomenon often reported in the literature [44,45]. The relatively low values of N concentration in the leaves, when compared to the sufficiency range of okra [46], suggest that the amount of N provided by guano enhanced plant growth, but it was not enough to increase the concentration of the nutrient in the tissues. The interpretation of the results of the N bioavailability to plants becomes easier when the apparent recovery of the nutrient is taken into account (Table 5). Plants from some treatments recovered more N than that applied as a soil amendment (values greater than 100%) and the amount of N recovered was not directly related to the rate of guano applied. In most agro-systems, apparent N recovery tends to be low, typically between 40 to 60% [34,35] and generally decreases as the rate of N applied increases [43,47,48]. In this study, there seems to be a tendency for higher values to be associated with more productive treatments. It seems that the application of guano enhanced plant growth beyond its nutrient content. That is, plants with better growing conditions took up more N from native soil organic matter than plants in poorer growing conditions. The phenomenon is called 'priming' or 'added N interaction'

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[49,50] and has been reported in several other studies [36,51,52,53]. It seems that the use of fertilizers or organic amendments can stimulate biological processes in the soil that lead to an increase in the bioavailability of some nutrients.

Guano application significantly increased leaf P concentration in 2019 and the amount of P recovery in both years compared to the control. Bat guano often have high levels of P [54,55] and the same was observed for the guano used in this study (Table 3). The high P content in bat guano was probably the main reason for the greater effect of guano on P concentration than on N concentration in plant tissues. APR often exceeded 20%, values that can be considered high, taking into account that several reactions related to soil pH can quickly fix P in relatively unavailable forms [34,56]. Thus, it was probably the intense mineralization of the organic substrate during the growing season, stimulated by a warm climate and a well-aerated sandy soil, which regularly supplied P to the plants and ensured relatively high APR values. However, the values were well below 100%, meaning that much of the P remained immobilized in the soil. Given that these soils are limestone-derived, the decrease in P availability in the soil was probably due to the presence of Ca. In the presence of calcium carbonate, soluble P (monocalcium phosphate) rapidly evolves into a sequence of lower solubility products such as dicalcium phosphate and then tricalcium phosphate [35,36].

The application of guano did not significantly influence leaf K concentration and the highest K recovery values were associated with the most productive treatments. AKR values were particularly high in 2018, having exceeded 400% in some treatments. In 2019, the highest AKR values were related to the most productive treatments. This means that most of the K came from the soil and not from the organic amendment. If on the one hand this can be seen as a positive aspect, as it means that the soil itself can provide large amounts of K, it will still be of importance in a long-term fertilization strategy, as the crop will progressively remove K in a way which may not be sustainable. Nutrient mining is a relevant issue for agro-systems, as it corresponds to a negative balance between nutrient input through fertilizers and manure, and output through crop removal [57]. This is of particular concern in sub-Saharan Africa, due to the difficulties for smallholder farmers in accessing commercial fertilizers [58,59]. They should adopt whenever possible practices of crop rotation and intercropping as a way of mitigating the loss of soil fertility [12,13,60].

The results of B are also noteworthy. Leaf B concentration increased in guano treatments, suggesting again high mineralization of the organic amendment. As the guano mineralized, the nutrient was released in sufficient quantity to change its concentration in plant tissues. Thus, the results of B were somewhat similar to those of N, perhaps also due to the close relationship between B availability and the dynamic of organic matter in the soil [61]. Local conditions, however, seem to provide high amounts of this nutrient to plants, since leaf B concentration in the control was found close to the upper limit of the sufficiency range [46], indicating that B is not a major concern in crop fertilization.

4.3. Biochar increased total soil organic carbon and guano the easily oxidizable carbon

Soil amendment treatments significantly affected soil C content. Total organic C (incineration) was more affected by biochar applications whereas easily oxidizable C (Walkley-Black) by guano applications. Biochar is difficult to decompose, due to a molar H/C ratio generally below that of the feedstock, which indicates polymerization and therefore potential recalcitrance [38]. This justifies its presence in the soil at the end of the growing season, being the direct result of its addition to the soil and a reduced rate of mineralization. In agreement with this, many previous studies have shown the high capacity of biochar to contribute to C sequestration in the soil [37,38,39,43,62]. Data on nutrient uptake by okra suggests a high mineralization of guano, probably due to the high temperature in the region and the well-aerated soil in which the study was carried out. Thus, the increase in easily oxidizable C would not have been due to the applied guano, as has been reported in other studies [17,18], but perhaps more to the products of photosynthesis, which can be deposited in the soil by plant roots and mycorrhizal fungal mycelia [53,63,64]). That is, the increase in easily oxidizable C observed in the soil at the end of the season was mainly due to crop residues, which were greater in the most productive treatments, those that received guano, and less to the C initially contained in the organic amendment, which would have mostly been mineralized.

Soil P levels also varied significantly with treatments. However, the values do not seem to have varied in the same way in the two growing seasons. There seems to be a pattern of high values in the treatments that received higher rates of guano, perhaps due to their initial P content, as already mentioned, and to the high turnover of organic C in the most productive treatments, since the P cycle is also linked to the dynamics of organic matter in the soil [34,35,56]. Biochar treatments did not increase P availability in the soil. Some previous studies refer to an increase in the availability of P in the soil by the application of biochar, the results being justified by the increase in the anion exchange capacity [38,65] or by the reduction in the formation of precipitates of P either in acidic or alkaline soils, which leads to increased P availability to plants [66,67]. However, as in both this and several other studies, no increase in P availability was observed after the application of biochar [42,44,62].

The application of biochar increased the CEC in relation to the control treatment, with Ca being the exchange base with the greatest contribution to the result. Biochar has high pH and Ca content (Table 3), which may explain the result. On the other hand, biochar contains amorphous aromatic compounds with heteroatoms in the aromatic rings, which play a great role in making the surface of biochar heterogeneous and reactive [38], often leading to an increase in CEC after applying biochar to soil [37,38,43,62]. The application of guano also caused a slight increase in CEC, perhaps because crop residues led to an increase in easily oxidizable C and, as is known, organic matter has a very high CEC [34,35].

4.4. Potted cabbage showed low residual effect for guano and nil for biochar

Cabbage grown in pots, and used as a soil residual fertility index following the harvesting of okra, resulted in higher DMY in 2019, contrary to what had been recorded with okra height and fruit yield, whose average values had been higher in 2018. The environmental variables that limited okra crop growth in 2019 led to better soil residual fertility, allowing for an increase in cabbage DMY. In 2019, significant differences were found between treatments in tissue N concentration, with the higher values being associated with treatments that received higher rates of guano. The result tended to be repeated for P and also for B, but not for K, for which no significant differences were recorded between treatments. In previous studies, the use of plants as a biological index of nutrient availability in the soil has shown good reliability [68,69,70]. In this case, it contributed to show a reduced residual effect of the guano application, which must have been largely mineralized during the okra growing season, as observed by Dimande et al. [36] in a previous study with maize in similar agro-ecological conditions. The pot experiment also showed that the application of biochar did not significantly influence any of the measured variables related to nutrient uptake and plant growth compared to the control treatment.

5. Conclusions

The application of guano increased plant growth and yield. Its effect was due to the release of important nutrients such as N and P, but probably also to a certain "manuring effect". That is, the application of the organic amendment may have had a positive effect on the plants in addition to its nutrient content. The application of guano enhanced plant growth and yield and this led to increased uptake of some important nutrients in relation to the amount of nutrient contained in the organic amendment itself. Tissue nutrient concentration, apparent nutrient recovery, some soil properties and the residual effect of fertilization assessed by potted cabbage, collectively suggest that guano underwent intense mineralization during the growing season, probably due to its low C/N ratio, the high temperatures of the region and the well-aerated loamy-sand soils where the study took place. Early application of guano did not provide more benefits than applications close to sowing, perhaps due to the rapid mineralization undergone by the guano. The use of biochar significantly affected neither okra growth and yield nor tissue nutrient concentration, compared to the unfertilized control. However, the use of biochar increased the total organic C in the soil and the CEC, aspects that can benefit long-term cultivation processes.

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