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Under a Tropical Climate and in Sandy Soils, Bat Guano Mineralises Very Quickly, Behaving More like a Mineral Fertiliser than a Conventional Farmyard Manure

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Abstract: In sub-Saharan Africa, soil fertility management must rely on local fertiliser resources since most smallholder farmers do not have access to industrial fertilisers. In Vilankulo, Mozambique, farmers have access to bat guano and biochar, albeit in small amounts, which makes it even more necessary to manage them correctly to maximise crop productivity. This study was carried out with irrigated maize (*Zea mays* L.) in a haplic Lixisol during the 2017/2018 and 2019 growing seasons. Nine treatments were established consisting of the application of 5 (G5) and 10 (G10) t ha^{-1} of guano at sowing, 5 (B5) and 10 (B10) t ha^{-1} of biochar at sowing, 5 [G5(-1)] and 10 [G10(-1)] t ha^{-1} of guano one month before sowing, 1 and 4 (B1G4) and 2 and 8 (B2G8) t ha^{-1} of biochar and guano, respectively, at sowing and an unfertilised control (C). Treatments G10 and B2G8 led to the highest maize yields (3.77 and 2.68 t ha^{-1} in 2018 and 5.05 and 5.17 t ha^{-1} in 2019, respectively), and were statistically higher than those of the control (1.35 and 1.63 kg ha^{-1}, respectively). Apparent nitrogen recovery from bat guano was close to 100%, showing almost complete mineralisation during the maize growing season, due to its low carbon/nitrogen ratio and very favourable environmental conditions for mineralisation. Due to the fast release of nutrients, bringing forward the application of the organic amendment before sowing is not recommended, since it reduces nutrient use efficiency. Biochar did not significantly influence maize grain yield or contribute significantly to plant nutrition. To take advantage of its potential effect on some soil properties, its use in combination with other materials of greater fertilising value is recommended.

Keywords: conservation agriculture; soil fertility management; sub-Saharan Africa; maize yield; biochar; bat droppings

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1. Introduction

Two of the most important challenges regarding global food security are the continuously increasing human population, which is expected to reach 9.7 billion in 2050 and 10.4 billion in 2100 [1], and the impact of climate change on food, fibre, and other ecosystem products [2]. Furthermore, most agro-systems are experiencing some kind of threat to soil fertility, such as acidification, salinisation, the reduction of organic matter, and/or nutrient depletion, which makes soils less productive [3,4]. To ensure sustainability in food production for a growing world population, and to enable adaptation to a changing climate, it is necessary to adopt agro-ecological practices that increase soil fertility and reduce environmental contamination [5]. The use of organic amendments is commonly seen as one of these practices, as it contributes to climate change mitigation by sequestering carbon (C) in the soil and reducing emissions into the atmosphere [6,7].

In several African countries, the use of organic amendments is even more important due to the difficulty farmers have in accessing industrial fertilisers [8]. The use of organic amendments plays an important role in soil conservation strategies, due to their effect on soil biological activity, restoration of soil organic matter, and the increase in nutrient cycling [9–11]. The use of manures, together with crop rotation and intercropping, is the basis of the "Integrated Soil Fertility Management" concept [12] and is consistently mentioned as the most recommended practice for small farmers of Sub-Saharan Africa [13–15].

Bat guano has been mined for a long time in different countries and used as a biofertiliser due to its high content in macro- and micronutrients [16,17]. Bats are present on all continents, except Antarctica, with about 1300 species, which represent 20% of mammalian taxa [18]. Thousands of bats leave their droppings in the caves in which they live, where they accumulate over centuries, harden and turn into guano [19]. The mineral composition of guano is very variable, depending on the species of bats and their main diet, as well as on whether the caves they inhabit are located in dry or humid climatic zones [20]. Nevertheless, several studies have demonstrated a positive effect of bat guano on soil fertility and plant nutrition [19,21,22].

Biochar, a C-rich product obtained from the burning of biomass under conditions of reduced oxygen supply, has been intensively investigated all over the world as an improver of soil properties and for its recalcitrance and ability to sequester C in the soil [23–26]. Biochar can persist in soils for hundreds or thousands of years and, by increasing soil pH, porosity, and water availability, can create favourable conditions for the development of plant roots and the growth of microbial communities [25]. Biochar can be produced by simple artisanal processes, using local organic resources from agricultural and forestry activities as feedstock [27], which can be a useful way of recycling those materials and having a positive impact on a smallholder's income.

In tropical sandy soils, strategies to conserve organic matter and reduce nutrient mining are even more necessary than in soils in temperate regions with higher clay content. The annual removal of nutrients in crops, such as nitrogen (N) and potassium (K), is frequently greater than the natural capacity of the soil to replace them, with continuous depletion occurring as a result [9,28]. To attain sustainable crop production, fertilisers, and manures must balance the annual removal of nutrients. Mozambique is a country where most farmers cannot access industrially synthesised fertilisers. According to FAOSTAT [29], the average consumption of N fertilisers in Mozambique, between 2009 and 2019, was 4.31 kg ha^{-1} $year^{-1}$, substantially lower than the average for Southern African countries, which stood at 30.96 kg ha^{-1} $year^{-1}$. Ref. [8] analysed the dynamics of the fertiliser value chain in Mozambique and found that key supply-side constraints to fertiliser use include high transaction costs, limited access to finance, and lack of soil testing and fertiliser recommendations by soil type and crop.

In the Vilankulo district, in southern Mozambique, natural guano is available and local farmers have mastered an artisanal technique of preparing biochar from agricultural and forestry residues. In this region, the soils are sandy or sandy loams, typical of tropical regions. Given the difficult access to industrially synthesised fertilisers by farmers, it is particularly important to assess the effect of these local organic resources on soil and crops and learn how to manage them to increase the sustainability of cropping systems.

Thus, in this study, the effects of bat guano and biochar applied at two rates (5 and 10 t ha^{-1}) on maize were evaluated. Guano was also mixed with biochar in two different combinations (guano 4 t ha^{-1} and biochar 1 t ha^{-1}, and guano 8 t ha^{-1} and biochar 2 t ha^{-1}). Guano was also applied at 5 and 10 t ha^{-1} one month before sowing. The experimental design also included an unfertilised control. Three main hypotheses were raised: (i) guano and/or biochar applications benefit soil properties and/or maize productivity; (ii) by mixing guano and biochar, a synergistic effect is achieved that cannot be obtained using the products on their own; and (iii) the application of guano one month before sowing improves the efficiency of nutrient use in relation to its application at sowing, by anticipating the beginning of the mineralisation process.

2. Materials and Methods

2.1. Site Description

The field experiments were conducted at the Joint Aid Management Life (JAM-Life) site (21°59′05″ S, 35°09′39″ E, Pambara Administrative Post) in Vilankulo district, southern Mozambique, over two consecutive growing seasons, 2017/2018 and 2019. Maize was grown each year on different plots within the farm and the grain was used for a program to supply maize porridge to local schools. The local climate, under the Köppen classification, is Aw (dry winter), corresponding to a tropical wet and dry climate or tropical savanna climate [30]. Vilankulo has two main seasons: the hot and rainy season, which runs from October to March, and the dry and cool season, which runs from April to September [31]. Long-term average annual precipitation rates and temperature at Vilankulo are 677 mm and 24.2 °C, respectively. The monthly precipitation rates and mean air temperature are shown in Figure 1 [32].

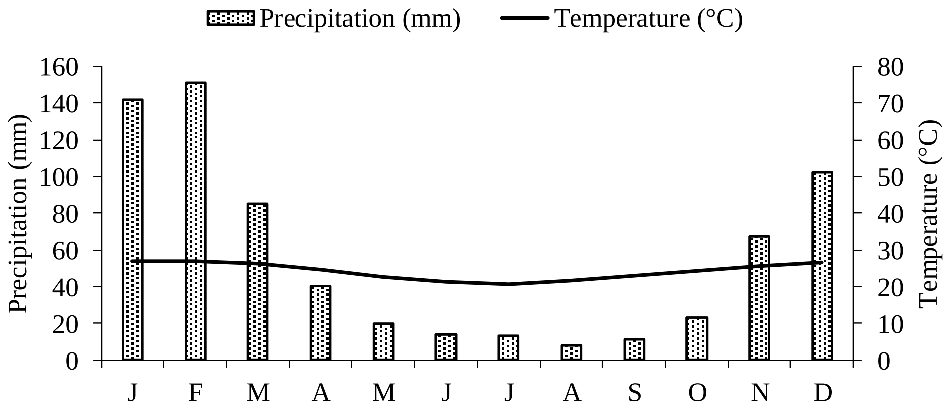

Figure 1. Average monthly precipitation and temperature in Vilankulo (1991–2021).

Under the classification of FAO [33], the soil is a haplic Lixisol, a well-weathered soil derived from limestone. Some soil properties of the plots where the experiments were carried out, determined from samples taken at 0–0.20 m depth at the beginning of the experiments, are shown in Table 1.

Table 1. Selected soil properties (average ± standard deviation, n = 3) from composite samples (10 cores per composite sample) taken at 0–0.20 m depth at the beginning of the study.

Soil Properties	2017/2018	2019
[1] Organic carbon (g kg^{-1})	3.9 ± 0.30	9.4 ± 0.86
[2] pH (H_2O)	6.8 ± 0.07	6.4 ± 0.16
[3] Extract. phosphorus (mg kg^{-1}, P_2O_5)	34.7 ± 10.17	38.0 ± 6.90
[3] Extract. potassium (mg kg^{-1}, K_2O)	97.2 ± 11.72	86.3 ± 16.24
[4] Exchang. calcium ($cmol_c$ kg^{-1})	2.8 ± 0.08	6.1 ± 0.72
[4] Exchang. magnesium ($cmol_c$ kg^{-1})	1.9 ± 0.17	2.4 ± 0.17
[4] Exchang. potassium ($cmol_c$ kg^{-1})	0.3 ± 0.03	0.2 ± 0.03
[4] Exchang. sodium ($cmol_c$ kg^{-1})	0.6 ± 0.12	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})	5.7 ± 0.07	9.5 ± 0.50
[7] Sand	91.0 ± 0.60	83.7 ± 0.98
[7] Silt	1.1 ± 0.21	5.2 ± 0.50
[7] Clay	7.9 ± 0.70	11.1 ± 1.17
[8] Texture	Sandy	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; [8] USDA (The United States Department of Agriculture).

2.2. Experimental Design and Organic Amendments

The experiments were arranged as a completely randomised design with nine treatments and three replicates. The treatments consisted of the 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 unfertilised control (C).

The organic amendment named guano in this study is bat excrement, locally available from natural deposits in Mapinhane Administrative Post and commonly used by farmers as a soil amendment. The elemental composition of the guano used in the two-year study is shown in Table 2. Biochar is also a material prepared locally from forest residues, collected in sawmills, and also from natural vegetation. Biochar was prepared through an artisanal method of slow pyrolysis, which consists of heating biomass waste (feedstock) into a reactor (made from two metallic drums) for one day. The reader is referred to [27] for more details on this type of slow pyrolysis. Some properties of biochar are shown in Table 2. The rates of the macronutrients N, P, and K applied in this study, taking into account the amounts of guano and biochar used in each treatment and their elemental composition are shown in Table 3.

Table 2. Selected properties (average ± standard deviation, n = 3) of the guano and biochar used in the experiment reported to dry matter.

	Guano		Biochar	
Properties	**2017/2018**	**2019**	**2017/2018**	**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})	4.5 ± 0.55	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})	28,188.0 ± 2720.97	45,606.2 ± 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.

Table 3. Nitrogen, phosphorus, and potassium application taking into account the amounts of guano and biochar used in each treatment and their elemental composition [G, guano; B, biochar; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing].

	Nitrogen (kg ha^{-1})		Phosphorus (kg ha^{-1})		Potassium (kg ha^{-1})	
Treatments	**2018**	**2019**	**2018**		**2019**	
G5	20.5	19.3	45.9	38.6	13.2	17.9
G10	40.9	38.6	91.8	77.2	26.4	35.9
B5	10.6	16.5	2.6	3.0	11.6	13.2
B10	21.3	33.1	5.2	6.0	23.2	26.4
G5(-1)	20.5	19.3	45.9	38.6	13.2	17.9
G10(-1)	40.9	38.6	91.8	77.3	26.4	35.9
B1G4	20.6	22.1	37.8	32.1	15.2	19.6
B2G8	41.2	44.1	75.4	64.2	30.4	39.3

2.3. Management of the Field Experiments

Soil preparation was done mechanically with a disc plough followed by a disc harrow at the beginning of the hot season in October. The subsequent operations of sowing, fertilisation, weed control, and harvesting were done by hand. The application of organic amendments and sowing was performed during November and December. The organic compost (guano) was applied on two dates depending on the treatments, as mentioned in the experimental design, either one month before or at sowing. In the season of 2017/2018, the organic compost was applied on 11 November and 11 December 2017, and the biochar on 11 December. Sowing was also done on 11 December 2017. Crop harvest was undertaken on 7 April 2018. In the growing season of 2018/2019, the amendments were applied on 8 February and 8 March 2019, sowing on 8 March 2019, and harvest on 15 July 2019. Maize seeds (cv. MRI 514) were spaced at 0.77 m between rows and 0.30 m within rows in a field where maize had been the preceding crop. The crop was grown under drip irrigation with an allocation of ~3000 m^3 per growing season. Although in the first year, the growing season took place in a normally rainy period, that year was very dry and the amount of water used was equivalent to that of 2019.

2.4. Field Determinations

Plant height, stem diameter, leaf number, and phenological stages [BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) scale] [34], were assessed periodically during the growing seasons. However, only plant height measurements were included in the results section. Plant height was determined with a tape measure from the ground to the highest tip of the highest leaf.

Leaf samples were collected during stem elongation when 9 or more nodes were detectable in the phenological stage 39 from the BBCH scale [34]. These were taken for elemental analysis and monitoring of the nutritional status of the plants. The procedure consisted of sampling eight fully expanded leaves with a collar below the whorl.

Harvesting was carried out manually at the phenological stage 89 when the maize was fully ripe and the grains were hard and shiny. The grain was separated from the husk and the husk was added to the straw to obtain the grain yield as distinct from the total aboveground biomass.

2.5. Pot Experiment

At the end of the field trial, the soil was sampled for determination of general soil properties, and additionally to carry out a pot experiment to obtain a biological index of soil nutrient availability. After the harvest of maize, soil samples were taken per experimental unit from the 0–0.20 m layer. The soil was air-dried and sieved (2 mm mesh). A subsample was used for the soil property determination and the remaining soil was used for the pot experiment. The plant used in this experiment was cabbage (*Brassica oleracea* L., cv. Tronchuda). The pots were filled with 2.5 kg of soil and placed in an open space. The pots were protected from direct solar radiation with newsprint to prevent the overheating of the rhizosphere. In the season of 2017/2018, cabbage was grown from 1 July to 16 August 2018, whereas in the season of 2018/2019, it was grown from 29 August to 15 October. During the experimental period, whenever precipitation was insufficient, the pots were watered applying 100 mL of water per pot. At phenological stage 18, corresponding to eight or more true unfolded leaves [34], the plant was cut at ground level, oven-dried at 70 °C, and weighed. Then the plants were ground (1 mm mesh) for elemental analysis.

2.6. Laboratory Analyses

Soil samples were analysed for pH (H_2O and KCl) (soil/solution, 1:2.5), cation-exchange capacity (ammonium acetate, pH 7.0), total organic C (incineration), easily oxidisable C (wet digestion, Walkley-Black) and extractable phosphorus (P) and K (Egner-Riehm method). Soil boron (B) was extracted by hot water and determined by the method of azomethine-H. Soil separates were determined by the Robinson pipette method. For more details on these analytical procedures, the reader is referred to [35]. The availability of other micronutrients (copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn)) in the soil was determined by atomic absorption spectrometry after extraction with DTPA (diethylenetriaminepentaacetic acid) buffered at pH 7.3, following the standard procedure of FAO [36].

Tissue samples (maize grain, leaves and stalks, and whole cabbage) and guano samples were oven-dried at 70 °C until a constant weight was obtained, 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 [calcium (Ca), magnesium (Mg), Cu, Fe, Zn, and Mn] methods, after nitric digestion of the samples [37]. Guano samples were also analysed for total organic C (incineration) and pH (guano/solution, 1:2.5) [35].

2.7. Data Analysis

Results were analysed for normality and homogeneity of variance using the Shapiro–Wilk and Bartlett's tests, respectively. The statistical software SPSS Statistics (v. 25, IBM SPSS, Chicago, IL, USA) was used to perform ANOVA (one-way ANOVA) and compare treatments. When significant differences were found, the post hoc Tukey HSD test (α = 0.05) was used for mean separation. Apparent nutrient recovery was estimated according to the following equation:

$$\text{Apparent nutrient recovery (\%)} = 100 \times [(\text{Nutrient recovered in the amended treatments} - \text{Nutrient recovered in the control treatment})/\text{Nutrient applied as an organic amendment}].$$

3. Results

3.1. Maize Dry Matter Yield and Plant Height

Maize grain yield varied significantly ($p < 0.05$) between treatments in both years (Figure 2). The application of guano at sowing, mainly at 10 (G10) and 8 (B2G8) t ha^{-1}, showed the highest average values between all treatments, especially when analysing the results of the two years together. The sums of grain yields for the two years were 8.82 (G10) and 7.85 (B2G8) t ha^{-1}, while in the control only 2.98 t ha^{-1} were recorded. Guano applied one month before sowing did not increase grain yield to the level observed with guano application at sowing. Mean values, however, were higher (4.62 t ha^{-1} in the G10(-1) treatment) than those observed in the control treatment. The use of biochar alone also did not present significantly higher values than those of the control, although the mean accumulated values were higher (4.48 t ha^{-1} in the B10 treatment).

Total maize dry matter yield followed a similar pattern to grain yield (Figure 3). The most productive treatments were those corresponding to the highest rates of guano applied at sowing. Accumulating the values over the two years, in treatments B2G8 and G10, 24.6 and 22.2 t ha^{-1} were obtained, while in the control only 12.8 t ha^{-1}.

Treatments with guano applied one month before sowing and with biochar applied alone did not give total dry matter yields significantly different from those of the control.

The plants from the treatments that produced less grain and biomass did not grow as much as the most productive ones (Figure 4). Control treatment plants were significantly smaller than those of the treatments G10 and B2G8, which were also the most productive. In 2019, plants from the control treatment were significantly smaller than those of any other treatment. Overall, plant height followed a pattern not dissimilar to grain yield and total biomass.

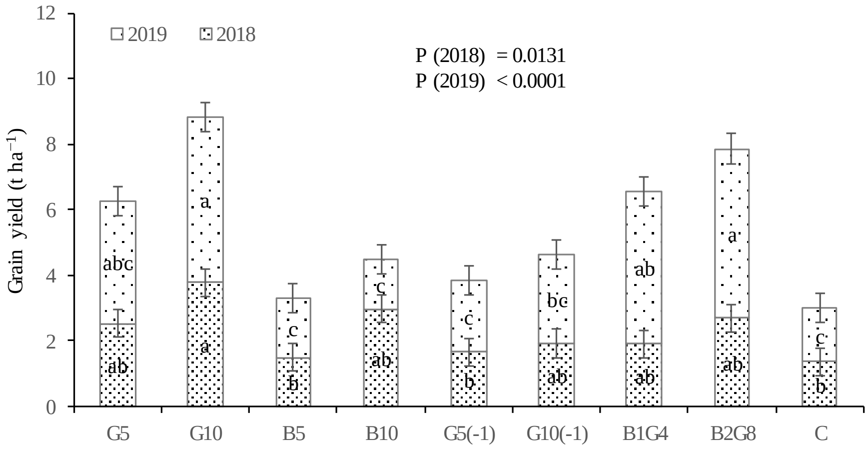

Figure 2. Maize grain yield as a function of fertilisation treatments (G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing). To each year, means followed by the same letter are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars are the standard error of the mean (n = 3).

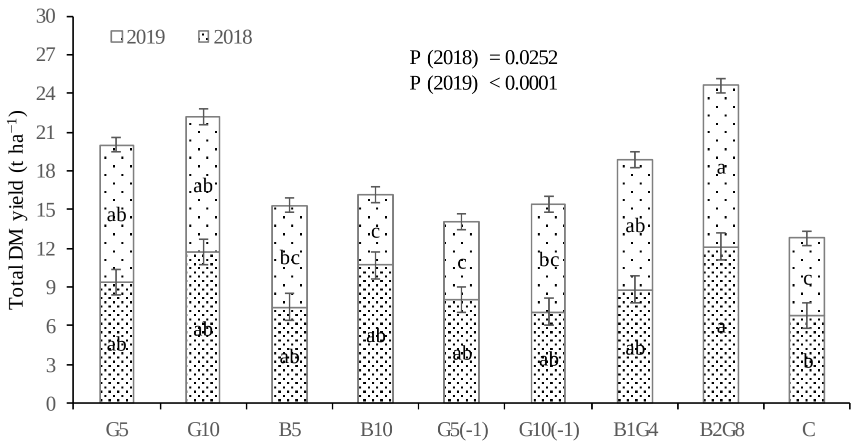

Figure 3. Total dry matter yield as a function of fertilisation treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing]. To each year, means followed by the same letter are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars are the standard error of the mean (n = 3).

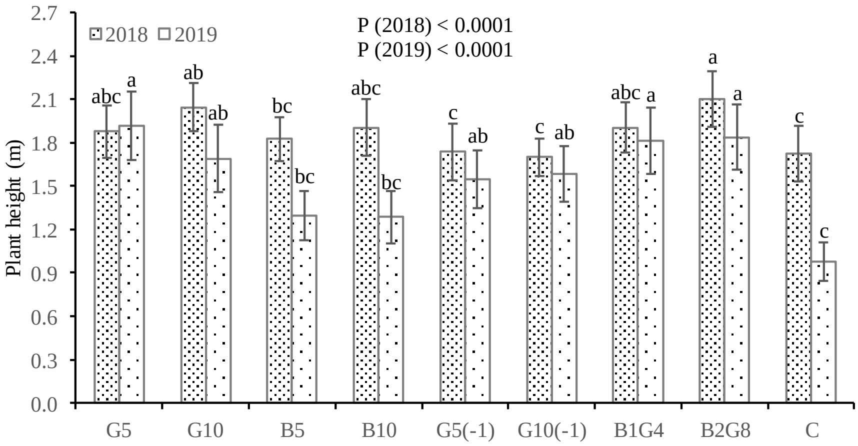

Figure 4. Maize plant height as a function of fertilisation treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing]. To each year, means followed by the same letter are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars are the standard error of the mean (n = 3).

3.2. Plant Nutritional Status and Residual Effects of Applications of Amendments

In 2018, significant differences were observed between treatments in N concentration in maize leaves (Figure 5). The control and the treatment corresponding to the application of 5 t ha^{-1} of biochar (B5) recorded the lowest average values (20.8 and 20.3 g kg^{-1}, respectively) while the B2G8 treatment displayed the highest average value (25.9 g kg^{-1}). In 2019, no significant differences between treatments were found in leaf N concentration and mean values ranged between 23.7 (C) and 29.1 (B2G8) g kg^{-1}. Leaf P, K and Ca concentrations did not vary significantly between treatments in either of the two years of the study. In 2018 and 2019, the average levels of P in the leaves varied over the ranges of 2.7–3.0 g kg^{-1} and 2.1–2.9 g kg^{-1}, respectively. Average K levels varied between 15.4–18.9 g kg^{-1} and 20.5–25.5 g kg^{-1} and Ca levels between 1.6–1.9 g kg^{-1} and 1.6–2.7 g kg^{-1}. Leaf Mg levels varied significantly between treatments in 2019. In 2018 average values varied between 1.2–1.4 g kg^{-1}. In 2019, the higher values (2.5 g kg^{-1}) were found in the G5(-1) treatment and the lower ones (1.7 g kg^{-1}) in the control. Leaf concentrations of the micronutrients B, Fe, Cu, Zn, and Mn were also determined in this study, but no significant differences between treatments or any consistent trends worth reporting were recorded, despite the extraordinarily high values of Fe in the biochar, 28.1 and 45.6 g kg^{-1} in 2018 and 2019 respectively; these are much higher values than any of the macronutrients (Table 2).

The dry matter yield of cabbage grown in the pot experiment was used as a biological index of soil nutrient availability and as a measure of the residual effect of the application of organic amendments. The results showed that there were no significant differences between treatments in any of the years (Figure 6). The treatments corresponding to the highest rates of guano applied at sowing, which had registered greater biomass and grain yields, did not maintain the same results in the potted cabbage. Tissue nutrient concentration and nutrient recovery by aboveground biomass also did not show significant differences or any consistent trends between treatments.

3.3. Nutrient Removal in Maize Aboveground Biomass and Apparent Nutrient Recovery

The amount of nutrients found in the aboveground biomass is a function of the biomass produced (grain and straw) and its nutrient concentration. The results of nutrient removal in maize aboveground biomass are shown in Figure 7. N removal in maize plants varied significantly between treatments only in 2019. However, the pattern of the differences observed between treatments was similar to that observed for grain (Figure 2) and total dry matter yield (Figure 3). The control showed very low annual N removal values, slightly higher than 50 kg ha^{-1}, while in the B2G8 treatment, the average values were close to 100 kg ha^{-1}.

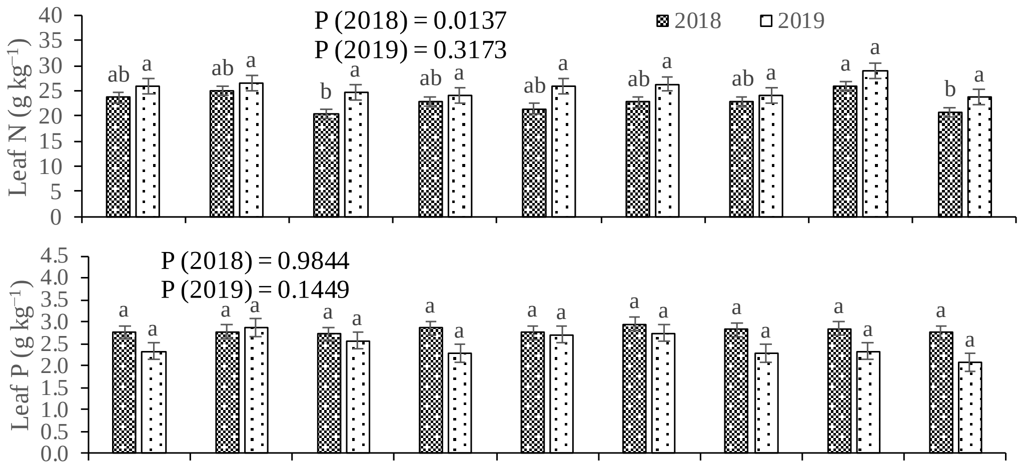

Figure 5. *Cont.*

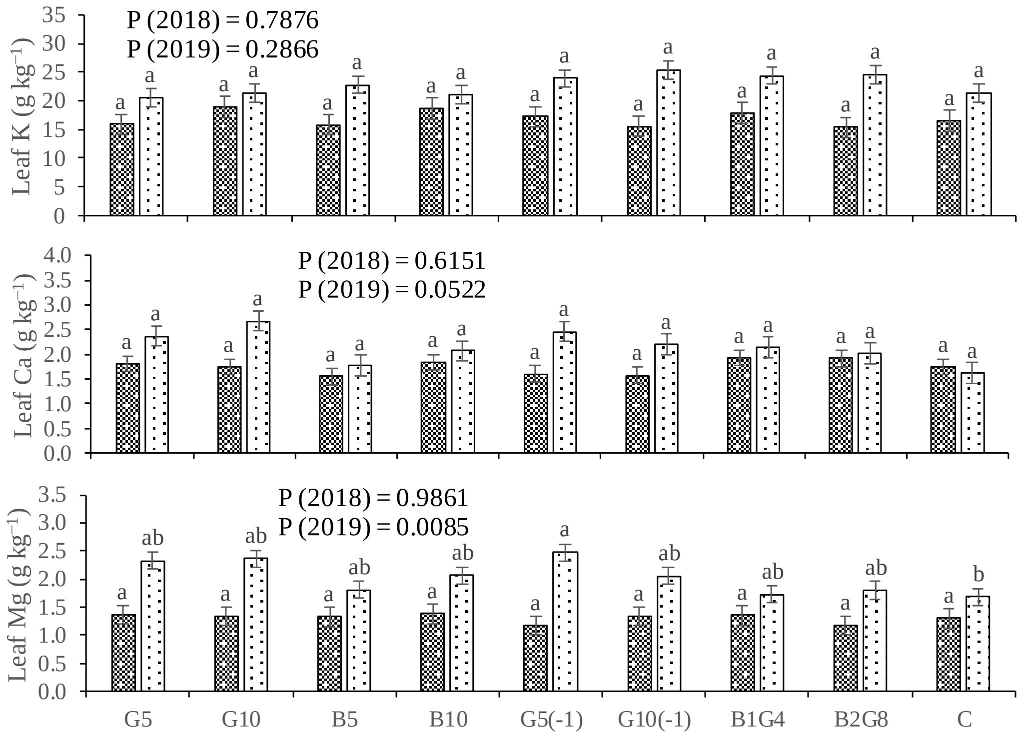

Figure 5. Maize leaf concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) as a function of fertilisation treatments (G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing). To each year, means followed by the same letter are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars are the standard error of the mean (n = 3).

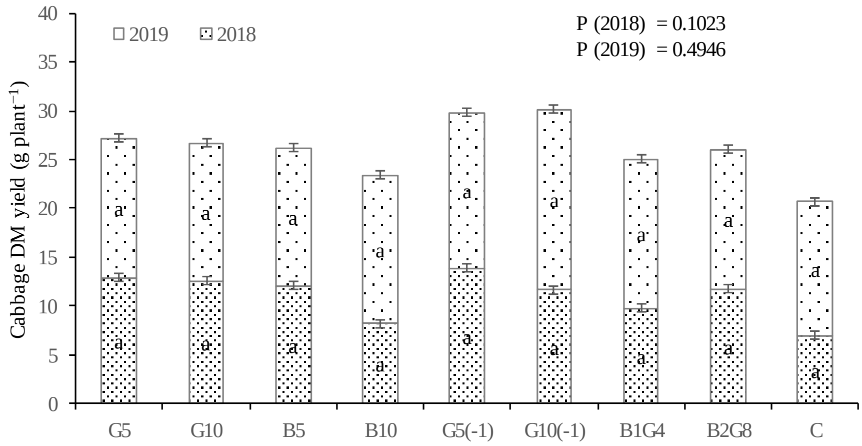

Figure 6. Cabbage dry matter (DM) yield in the pot experiment as a function of fertilisation treatments (G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing). To each year, means followed by the same letter are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars are the standard error of the mean (n = 3).

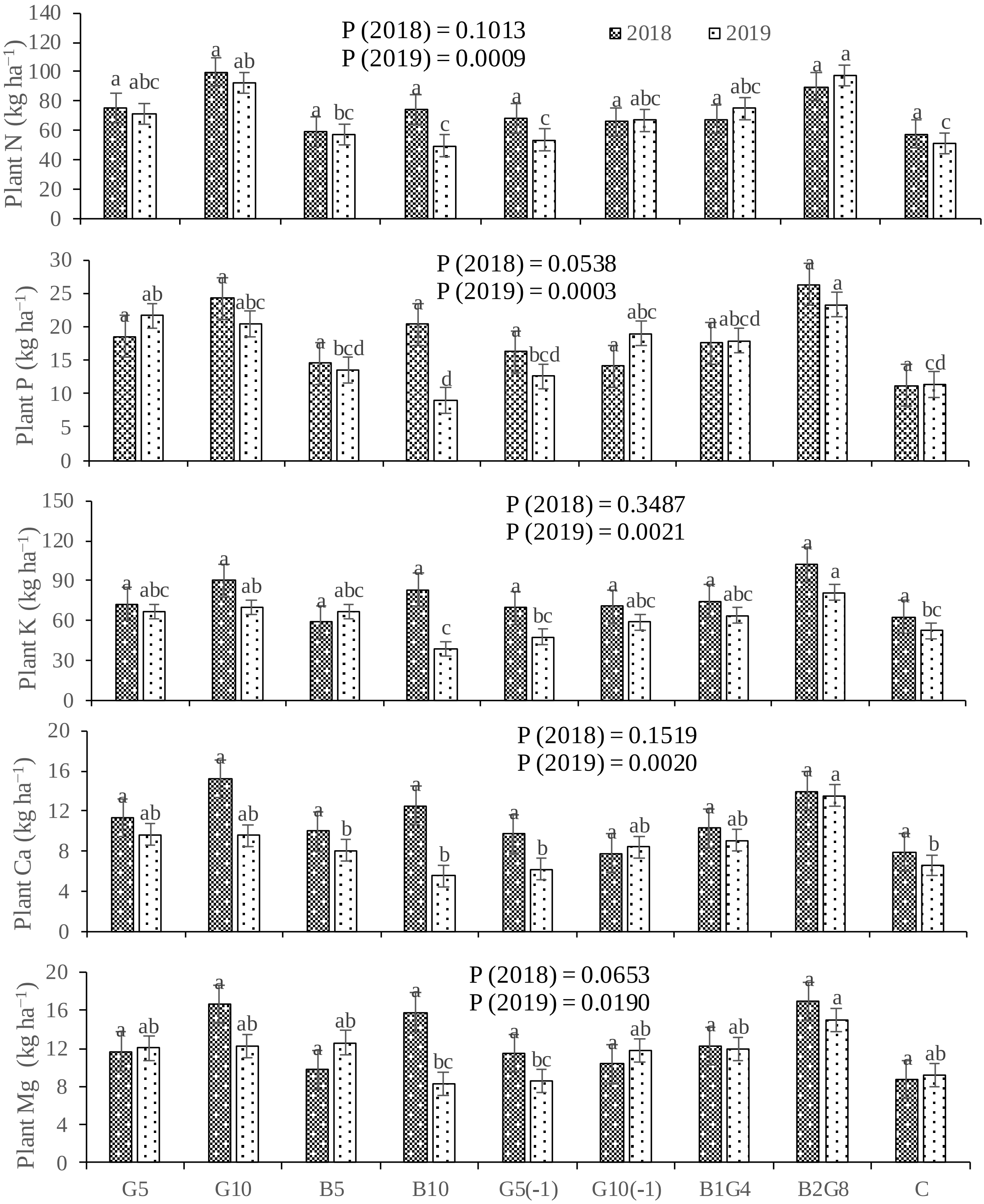

Figure 7. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) removal in aboveground maize biomass as a function of fertilisation treatments (G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing). To each year, means followed by the same letter are not significantly different by the Tukey HSD test (α = 0.05). Vertical bars are the standard error of the mean (n = 3).

Additionally, in the case of P removal from aboveground biomass, while significant differences between treatments were only observed in 2019 (Figure 7), the concentration of P in the leaves did not vary significantly between treatments (Figure 5), and nor did the concentration of P in the grain and straw at harvest. Thus, the results were mainly determined by differences in grain and straw production. Total P recovered in aboveground biomass varied, in any given year, from average values close to 11 kg ha^{-1} in the control to values close to 25 kg ha^{-1} in the B2G8 treatment. The pattern observed for K was similar to that observed for P and significant differences between treatments were only recorded in 2019. Overall, removal K values varied between 59 (B5) and 102 (B2G8) kg ha^{-1} in 2018 and between 39 (B5) and 81 (B2G8) kg ha^{-1} in 2019. The amounts of Ca and Mg removed in maize plants also only differed between treatments in 2019. Mean Ca values ranged between 8 (C) and 15 (G10) kg ha^{-1} in 2018 and between 6 (B10) and 14 (B2G8) kg ha^{-1} in 2019. The average values of Mg removed in the aboveground biomass of maize varied between 9 (C) and 17 (B2G8) kg ha^{-1} in 2018 and between 9 (B10) and 15 (B2G8) kg ha^{-1} in 2019. The removal of micronutrients, including Fe, did not vary significantly between treatments in any of the years.

The apparent recovery of N applied as guano at sowing was close to 100% (Table 4). When the organic amendment was applied one month before sowing, apparent N recovery dropped to values between 30 and 33%. Apparent N recovery from the biochar applications was also close to 30%. In treatments with a mixture of biochar and guano, the average values were between 78 and 92%. Apparent P recovery in treatments with guano applied at sowing was close to 20%, while in treatments with early application of guano, it was close to 7%. In treatments with biochar, it was high, between 68 and 101%. K values were high for guano applications at sowing (77%) and lower when guano applications were carried out one month earlier (15 to 25%). In the treatments with biochar, the average values varied between 19 and 38%. Ca recovery showed high values (90 to 120%) when guano was applied at sowing but with much lower values in the other treatments. Mg values showed a similar trend to Ca but with less marked differences between treatments.

Table 4. Apparent nutrient recovery by maize (average values of 2018 and 2019) as a function of fertilisation treatments (G, guano; B, biochar; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing).

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
			%		
G5	96.0	21.3	77.2	121.0	64.2
G10	105.1	13.0	77.2	90.0	63.8
B5	25.7	101.2	38.2	12.2	37.2
B10	36.5	68.7	19.1	6.6	30.9
G5(-1)	32.9	7.1	14.7	19.6	27.6
G10(-1)	30.6	6.5	24.9	18.8	22.7
B1G4	78.2	18.6	68.9	30.0	53.9
B2G8	91.5	19.3	102.0	39.9	60.9

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

3.4. Soil Properties

Total organic C and easily oxidisable C did not vary significantly between the treatments that received biochar and/or guano in any of the years (Table 5). However, in 2019 the control treatment showed significantly lower values of total organic C than the higher-yielding treatments (G5, G10, B2G8) or those that received biochar (B5, B10, B2G8). Soil pH also did not change with soil treatments either in 2018 or 2019. Extractable P, in turn, varied significantly between treatments in 2018, but not in 2019. Observing the results of the two years, the control treatment showed a trend towards lower mean values, a pattern similar to that observed for K, where significant differences between treatments were only found in 2018.

Table 5. Total organic carbon (TOC), easily oxidisable carbon (EOC), pH $_{(H_2O)}$, extractable phosphorus (P), and potassium (K) as a function of fertilisation treatments [G, guano; B, biochar; C, control; 5, 10, 1, 4, 2, 8, t ha^{-1}; (-1), applied 1 month before sowing] at the end of the maize growing seasons.

	TOC (g kg^{-1})		EOC (g kg^{-1})		pH $_{(H_2O)}$		P (mg kg^{-1}, P_2O_5)		K (mg kg^{-1}, K_2O)	
	2018	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
G5	8.7 a	12.2 a	4.0 a	10.2 a	6.6 a	6.2 a	21.1 b	49.1 a	108.3 ab	96.0 a
G10	9.0 a	12.3 a	3.9 a	10.4 a	6.8 a	6.2 a	51.0 ab	64.7 a	130.3 a	86.2 a
B5	9.4 a	13.0 a	4.0 a	11.4 a	6.9 a	6.5 a	33.4 b	29.2 a	83.3 ab	101.7 a
B10	9.5 a	13.5 a	4.1 a	12.3 a	6.8 a	6.5 a	33.5 b	75.1 a	100.7 ab	114.0 a
G5(-1)	8.6 a	11.2 ab	3.9 a	10.9 a	6.7 a	6.2 a	43.5 ab	86.4 a	103.3 ab	86.3 a
G10(-1)	9.1 a	11.2 ab	3.7 a	11.0 a	6.7 a	6.2 a	30.9 b	54.9 a	104.3 ab	101.3 a
B1G4	9.3 a	11.6 ab	3.7 a	11.1 a	6.8 a	6.4 a	31.1 b	63.1 a	83.7 ab	103.3 a
B2G8	9.4 a	13.0 a	4.2 a	11.0 a	6.9 a	6.3 a	81.1 a	66.8 a	105.7 ab	93.0 a
C	7.7 a	9.6 b	3.9 a	9.4 a	6.8 a	6.2 a	15.0 b	18.4 a	76.7 b	81.0 a
Prob > P	0.0713	0.0014	0.1301	0.9795	0.6878	0.0563	0.0011	0.1138	0.0379	0.2182
Std. error	0.38	0.52	0.13	1.69	0.11	0.08	8.16	15.45	9.94	8.41

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

Exchangeable Ca varied significantly between treatments in 2019, but not in 2018 (Table 6). In 2019, the highest average value was found in treatment B10 (8.88 cmolc kg^{-1}), which was significantly different from the average value found in treatment G5 (5.64 cmolc kg^{-1}). Exchangeable Mg and K also did not differ significantly between treatments, nor did exchangeable sodium and exchangeable acidity. CEC did not differ significantly between treatments, showing that the contribution of exchangeable Ca to CEC was not enough for this variable to present significant differences between treatments. Even so, the highest exchangeable Ca and CEC values seem to be associated with the treatment that received the highest rate of biochar (B10).

Table 6. Exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and cation exchange capacity (CEC) as a function of fertilisation 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	2.62 a	5.64 b	0.81 a	2.38 a	0.28 a	0.16 a	4.26 a	9.18 a
G10	3.01 a	5.91 ab	0.97 a	2.35 a	0.37 a	0.12 a	5.08 a	9.24 a
B5	3.17 a	7.61 ab	1.00 a	2.28 a	0.21 a	0.21 a	5.08 a	11.03 a
B10	2.91 a	8.88 a	0.94 a	2.10 a	0.23 a	0.18 a	4.82 a	12.02 a
G5(-1)	2.56 a	6.83 ab	0.88 a	2.33 a	0.28 a	0.16 a	4.24 a	10.16 a
G10(-1)	2.44 a	6.50 ab	0.90 a	2.14 a	0.26 a	0.17 a	4.14 a	9.75 a
B1G4	2.76 a	8.07 ab	0.90 a	2.58 a	0.22 a	0.20 a	4.39 a	11.66 a
B2G8	3.10 a	7.34 ab	0.98 a	2.36 a	0.27 a	0.16 a	5.03 a	10.63 a
C	2.94 a	6.07 ab	0.95 a	2.51 a	0.21 a	0.16 a	4.78 a	9.61 a
Prob > P	0.1319	0.0307	0.491	0.5722	0.0877	0.0884	0.2662	0.0715
Std. error	0.186	0.640	0.062	0.168	0.035	0.018	0.332	0.690

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

4. Discussion

4.1. Guano Applied at Sowing

The application of bat guano at sowing significantly increased maize grain and total dry matter yield compared to the control, with the treatments with the highest guano rates, 10 (G10) and 8 (B2G8) t ha^{-1}, showing the highest mean values between treatments. Although not as evident, plant height showed a similar tendency to grain and total dry matter yield. Organic amendments can increase crop productivity by improving several soil properties, sometimes referred to as the "manuring effect" [38,39], and/or by releasing nutrients to plants [6,7,40]. With the exception of K, which does not form part of organic structures [41], nutrients are released as the organic substrate is mineralised. The rate of mineralisation depends mainly on its C/N ratio, being higher when the C/N ratio is lower, and vice versa [6,42]. Sometimes it is considered that the dividing line between mineralisation and biological immobilisation for the first stages of decomposition of an organic substrate is ~20:1, with net immobilisation tending to occur above this value and net mineralisation below [43]. Organic C represents the source of energy for heterotrophic microorganisms and N is the raw material for protein synthesis [5]. The C/N ratio of guano used in this study was 13 and 14 in 2018 and 2019, respectively, a value at which net mineralisation is expected to occur. In addition, farmyard manures usually contain cereal straw, which is rich in cellulose, hemicellulose, and lignin, compounds that are difficult to decompose in the soil [43]. Bat guano probably does not contain such compounds since it is only animal waste, which also facilitates its decomposition.

In addition, several environmental variables also affect the rate of mineralisation. Among the most important, temperature stands out, and the higher the soil temperature, the higher the mineralisation rate [43,44]. The mineralisation of an organic substrate is primarily an aerobic process. The better the soil aeration, the faster the process occurs [43]. Soils with high sand content are normally well aerated [5,44,45]. Clay, on the other hand, provides protection against the degradation of organic matter also due to the formation of clay-humic complexes [5,43]. The soils of the plots where the study took place have a very high percentage of sand. Thus, under the conditions of this experiment, the low C/N ratio of guano suggests a high mineralisation rate that would also have been greatly favoured by the good mineralisation conditions (high temperature and sandy texture).

Mid-season leaf N concentration tended to be higher in treatments that received guano at sowing, and the total amounts of N, P and other nutrients recovered from the aboveground biomass were also significantly higher in those treatments than in the control. Apparent N recovery from treatments with guano applied at sowing was close to 100%, indicating more or less complete mineralisation of the organic amendment. Taken together, these results clearly indicate that guano provided nutrients in significant amounts to plants and seem to be in line with the results of other studies in which increases in crop productivity were observed by applying bat guano as a soil amendment [19,21,22].

Bat guano is a product particularly rich in Fe, with values for this micronutrient much higher than those of any macronutrient. However, there were no records of Fe increases in soils or plants resulting from the application of the organic amendment. Fe solubility is very dependent on soil reduction conditions, being higher in waterlogged soils [46,47]. Under the conditions of this study, mainly because it took place in soils with high levels of sand, and with good aeration, the application of guano, although very Fe-rich, did not contribute to a relevant increase in the bioavailability of this nutrient in the soil. Furthermore, micronutrient cations such as Fe are readily available in acidic soils but reduce their solubility as pH increases. With increasing pH, the ionic forms of the micronutrient cations change first to hydroxyl ions and finally to the insoluble hydroxides or oxides of the elements [5].

4.2. Guano Applied One Month before Sowing

Earlier applications of guano by one month resulted in significantly lower grain yield and total dry matter yield than in treatments with guano application at sowing. The objective of these treatments was to anticipate the mineralisation of the organic substrate so that a greater fraction of the nutrients would be available to the plants during the growing season. Considering the results obtained with the guano applied at sowing, there seems to be no doubt that this organic substrate is easily mineralised, due to the low C/N ratio and the probable low content of cellulose, hemicellulose, and lignin. Moreover, environmental conditions (high temperature, aeration, and soil moisture) were very favourable to organic matter mineralisation. This anticipation of the mineralisation process and release of nutrients in relation to sowing, which is followed by a period in which young plants have a lower nutrient uptake capacity, led to poor effectiveness in this fertilisation strategy. The application of organic amendments and sowing took place at the beginning of the rainy season, which, combined with the sandy texture of the soil, were favourable conditions for the leaching of mobile nutrients such as K and, mainly, N [48]. The presence of an organic substrate (providing electrons) and precipitation (providing anaerobic conditions, albeit temporary) may also have led to N losses by denitrification [49], although these losses may have been less important than leaching due to the high sand content of the soil which ensures aeration. Thus, the reduction in apparent N recovery in the earlier treatments, as opposed to those at sowing, would have been due to the loss of N to the environment. In global terms this is one of the most important findings of this study as it helps to clarify the best date for bat guano application, increasing nutrient use efficiency while minimising environmental risk, which are two important objectives of research in agronomy. In the case of P, it seems that rapid precipitation of the nutrient occurred after the mineralisation of the organic substrate, since apparent P recovery was much lower when applied earlier, due to the longer period between application and opportunity for root uptake. Some particular reactions related to soil pH can fix P in relatively unavailable forms. In acid soils, these reactions involve mostly Al, Fe, or Mn, either as dissolved ions, oxides, or hydrous oxides. However, these soils are derived from limestone and their reactions are slightly acidic. Thus, the most likely reason for the decrease in soil P availability was the presence of Ca. In the presence of calcium carbonate, soluble P (monocalcium phosphate) quickly evolve into a sequence of products of decreased solubility as dicalcium phosphate and then tricalcium phosphate [5].

4.3. Biochar Effect on Soil and Plants

Biochar used alone did not increase crop yield. Grain and total dry matter yield tended to be lower in biochar treatments compared to treatments that received higher amounts of bat guano. Biochar also did not increase nutrient concentration in maize leaves compared to the control treatment, nor was there a clear increase in nutrient recovery in aboveground biomass. It also did not increase the nutrient content in the soil, nor the organic C content, in comparison to the other amended plots, just a slight tendency to increase exchangeable Ca, and consequently a slight tendency to increase CEC, but without significant differences for the other treatments. Biochar is not usually used in agriculture for the purpose of providing nutrients. It is a material with high recalcitrance in the soil, being difficult to decompose due to the aromatic nature of the C resulting from pyrolysis [50,51], and is therefore used in agriculture mainly as a soil conditioner [24,52,53]. Positive effects from the use of biochar are expected to be obtained by improving soil properties such as permeability and water-holding capacity [54,55]. Soil aeration provided by biochar may also increase soil N retention, by stimulating microbial N immobilisation and reducing N losses by leaching and denitrification [56,57]. Biochar has also been shown to enhance microbial communities [58,59] and to immobilise soil contaminants such as heavy metals or harmful organic compounds [53,60]. In biochar-amended plots, the availability of P to plants is enhanced by the anion exchange capacity of biochar [53,61]. Perhaps it was this aspect associated with the very low amounts of P in the biochar that led to the high values of apparent P recovery in the treatments receiving biochar.

Under the conditions of this study, the beneficial effects of using biochar were not clear in the short-term almost certainly because the amounts in which it was applied were not very high and because it was applied only once. However, in 2019 the total organic C was significantly higher than in the control treatment and similar to the treatments with higher maize yield. It seems that the contribution of biochar was due to its own C and that of the most productive treatments to the surplus of C from photosynthesis introduced into the soil by plant debris. Other studies reported a beneficial effect of the use of biochar by enhancing soil biological activity [24,26]. In addition, it has been observed that the beneficial effects of biochar tend to manifest themselves mainly over the long term [26], which it was not possible to evaluate due to the short duration of this study. When the rate of guano was reduced in the treatments where it was mixed with biochar (treatments B1G4 and B2G8), the results were very similar to those of treatments G5 and G10. Perhaps biochar should be used in a mixture with products that release nutrients, trying to explore better the benefit that biochar can have in improving soil properties. It has been observed from other studies that combining biochar with other fertilising materials often presents synergisms that improve plant nutrition [62,63]. The use of biochar embodies the circular economy concept of using all agricultural by-products in the most convenient way. In this case, taking into account the context of low soil fertility and reduced access to fertilisers by small farmers, it may be interesting to use organic residues in the form of biochar. Although the use of biochar had no short-term effect on crop productivity, and this was one of the limitations of the study, the fact that it increased total organic C in the soil compared to the control in one of the years suggests medium- and long-term advantages of using by-products from the farm as biochar.

4.4. Residual Fertilising Effect of Soil Amendments

Cabbage grown in pots using soil sampled at the end of the maize growing season in each of the treatments resulted in dry matter yield, nutrient concentration, and nutrient recovery in aboveground biomass without significant differences between treatments. In this experiment, the performance of cabbage, grown under more controlled conditions, was used as a biological index of the availability of nutrients in the soil, since the uptake of nutrients by plants is more sensitive than the use of chemical extraction methods commonly used in the laboratory [39,64]. In this particular study, the procedure was intended to evaluate the residual effect of treatments applied to maize, based on the principle that organic materials have a long-term effect on crops due to a mineralisation process that can be more or less lengthy depending on its composition and environmental conditions [5,43]. Organic amendments are used in agriculture, taking into account the so-called "decay series", used to forecast the annual rates of mineralisation, which helps farmers to manage these resources [39,65,66]. The decay series represents the fraction of organic material that normally decomposes in each of the years following its application [43]. In general, the lower the C/N ratio, the greater the fraction of the organic substrate that decomposes in the first year and vice versa [6,42].

It should be noted that this study took place in a region of high temperatures and in plots with soil with a high percentage of sand, which favours aeration, two of the main factors that accelerate the mineralisation of organic substrates [5,43]. This study provided clear evidence that most of the nutrients in guano were released during the maize growing season. To substantiate this thesis, reference should be made not only to the positive effect of guano on maize productivity and the high recovery of nutrients compared to the control but also to the failure of the early application strategy, which may have contributed to excessive early mineralisation, reducing nutrient use efficiency and the residual effect of guano on the growth of cabbage. The fact that maize and grasses, in general, have a high nutrient uptake capacity, and rapidly reduce the availability of nutrients in the soil [67], may also have contributed to the poor residual effect of guano.

5. Conclusions

Under the environmental conditions in which this study took place, being a hot climate and sandy soils, the bat excrement used, with a low C/N ratio, behaved much more like an inorganic fertiliser than a conventional farmyard manure. The organic amendment seemed to be fully mineralised during the growing season of maize, releasing its nutrients, contributing to the increase in grain yield, and reducing the residual fertilising effect left in the soil. Accordingly, bringing forward the date of applying the organic amendment in relation to sowing is not recommended, as it reduces the efficiency of nutrient use and increases the risk of environmental contamination. Biochar used alone did not have a noticeable short-term positive effect on plant mineral nutrition or grain yield. Due to the potential increase in organic C, reported in 2019 in comparison to the control treatment, perhaps its use should be combined with mineral fertilisers or other materials that release more nutrients to take advantage of their potential beneficial effects on some soil properties.

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