# An organic-based amendments with biochar and rabbit urine improve maize (Zea mays L.) growth, physiology, and soil microbial activity in acidic soils of Cameroon

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## Abstract

Maize (*Zea mays* L.) production in Cameroon is constrained by progressive soil fertility decline, which limits the productivity of conventional cropping systems. Environmentally friendly organic-based amendments are increasingly sought as alternatives to costly and often poorly adapted mineral fertilization. This study evaluated the effects of Biochar enriched with rabbit urine, applied alone or combined with NPK, on the agronomic performance, physiology and soil microbial activity of maize grown on an acidic clay soil at Bafia, Cameroon. The trial followed a randomized complete block design with three replications and eight treatments: unamended control (T0), NPK 200 kg $ha^{-1}$ (T1), Biochar 2.5 t $ha^{-1}$ (T2), rabbit urine 2000 L $ha^{-1}$ (T3), rabbit urine 2000 L $ha^{-1}$ + NPK 200 kg $ha^{-1}$ (T4), Biochar 2.5 t $ha^{-1}$ + rabbit urine 2000 L $ha^{-1}$ (T5), Biochar 2.5 t $ha^{-1}$ + NPK 200 kg $ha^{-1}$ (T6), and Biochar 2.5 t $ha^{-1}$ + rabbit urine 2000 L $ha^{-1}$ + NPK 200 kg $ha^{-1}$ (T7). Growth variables (leaf number, collar diameter, plant height, and leaf area), physiological variables (soluble sugars, soluble proteins, total phenolics, and total chlorophyll) and soil microbial activity were recorded throughout the study. All amended treatments significantly ($P < 0.05$) outperformed the unamended control for every variable measured. The combination of rabbit urine with NPK (T4) produced the tallest plants (176.6 cm), the largest collar diameter (27.5 mm) and the highest soil microbial activity (3.45 μg $mL^{-1}$), while the Biochar + rabbit urine combination (T5) maximized soluble protein content (48.6 μg BSA-eq $g^{-1}$ FW) and the Biochar + NPK combination (T6) maximized total chlorophyll content (7.02 μg $mL^{-1}$) while minimizing phenolic accumulation (9.17 μg eq $g^{-1}$ FW), indicative of reduced metabolic stress. These results indicate that Biochar enriched with rabbit urine, whether or not combined with mineral NPK fertilizer, is a promising, low-cost and environmentally sound option for restoring soil fertility and improving maize productivity in tropical, acidic-soil environments such as those found in Cameroon.


## 1. Introduction

Maize (*Zea mays* L.) is a major cereal crop worldwide, alongside wheat and rice, because of its wide ecological adaptability and high starch content, which make it important for human consumption, animal feed, and agro-industrial uses [1, 2]. In Cameroon, production is only about 2.1 million tonnes despite its favorable agro-ecological potential [3]. This production remains below the estimated domestic demand of 2.7 million tonnes, leaving a structural deficit of around 600,000 tonnes that is attributed to a combination of socio-economic, edaphic, biotic and abiotic constraints. To close this gap, farmers tend to respond less by adopting yield-enhancing technologies than by extending cultivated areas and intensifying existing cropland use, notably by shortening or suppressing the traditional fallow periods that once allowed soil fertility to regenerate [4].

Rising demand for maize is thus placing growing pressure on natural resources and conventional cropping systems in Cameroon, intensifying cultivation practices that accelerate soil degradation, reduce productivity and increase vulnerability to climate variability [5]. This intensification is rarely matched by adequate fertiliser input or organic matter restitution: continuous cropping without sufficient nutrient replenishment causes a net loss of soil nutrients at each harvest, progressively depleting soil fertility and depressing yields over time [4]. Cameroonian soils are, moreover, often inherently poor in or increasingly depleted of nitrogen, phosphorus and potassium, the nutrients most critical to maize growth [6], which makes them particularly vulnerable to this dynamic. This trend is compounded by demographic pressure and permanent cultivation without adequate restitution of organic matter, making the maintenance of soil fertility a central challenge for Cameroonian agriculture [7].

Conventional mineral fertilization, although effective in the short term, is often costly, logistically constrained for smallholder farmers, and insufficient on its own to durably restore degraded soils, while climate change further increases the vulnerability of cropping systems. Innovative organic amendments therefore represent a promising alternative for simultaneously restoring soil fertility and sustaining crop productivity. Among these, Biochar is recognized for its capacity to improve the physicochemical properties of soils, notably porosity, cation exchange capacity and water retention [8], whereas rabbit urine constitutes a readily available, nitrogen-rich liquid organic input capable of supplying nutrients that support plant growth [9]. However, the combined effects of Biochar and rabbit urine on maize agronomic and physiological performance, and on soil microbiological activity, remain poorly documented under field conditions in Central Africa.

Rabbit urine is rich in nitrogen and also contains other nutrients that can promote plant growth and development, offering the possibility of reducing farmers' dependence on costly mineral fertilizer [10, 11]. Biochar, in turn, can improve water retention, organic matter content and micronutrient availability in amended soils [12, 13]. The combination of these two organic-amendments could therefore have a synergistic effect on soil physicochemical properties and on crop yields, with reported yield gains of 10–30% when organic and mineral amendments are combined [14]. Biochar combined with conventional mineral fertilizer has also been shown to increase maize yield by more than 10% relative to NPK fertilization alone [14], while Biochar's capacity to sequester carbon in soil rather than releasing it to the atmosphere adds an additional environmental benefit [15, 16].

Given this context, the synergistic potential of Biochar and rabbit urine, alone or combined with mineral NPK fertilizer, for improving soil fertility, maize agro-physiological performance and soil microbial activity deserves particular attention. The present study aimed to evaluate the effect of an organic amendment based on Biochar combined with rabbit urine on the agronomic and physiological performance of maize and on soil microbiological activity.

## 2. Materials and Methods

# 2.1. Study site

The trial was conducted in the school farm of the Faculty of Agronomy and Agricultural Sciences (FASA), Bafia Annex, University of Dschang. Bafia is the administrative center of the Mbam-et-Inoubou Division, located approximately 120 km from Yaoundé (4°37'–4°46' N, 11°6'–11°18' E), at a mean altitude of 470 m. The site has a bimodal, sub-equatorial (Guinean-type) forest climate, with a mean annual temperature of about 23–25°C, mean annual rainfall of 1455–2000 mm, and relative humidity generally above 80%. The soils are dominated by lateritic profiles with a thick weathering mantle that favors the formation of stony soils on slopes, over a plateau relief largely covered by savanna vegetation.

# 2.2. Plant and fertilizer material

The maize variety used was Kabamanoj F1, a short-cycle (75–80 days to green-ear harvest), yellow-grained hybrid obtained from a phytosanitary input shop in Bafia; it is adapted to the Centre Region and reported to tolerate fall armyworm attack, with a yield potential of about 10 t $ha^{-1}$.

Three fertilizing materials were used: (i) rabbit urine, applied twice (at the 2–3 leaf stage and at the 6–8 leaf stage, 1000 L $ha^{-1}$ per application); (ii) Biochar produced industrially from coffee husks at approximately 500°C by the company NetZero (Nkongsamba), applied two weeks before sowing; and (iii) mineral fertilizer consisting of urea (46% N) at 150 kg $ha^{-1}$, applied 40 days after sowing, and NPK 14-24-14 at 200 kg $ha^{-1}$, applied 15 days after sowing.

1. Rabbit urine was collected at the FASA farm in Bafia and fermented for 14 days. The rate of 1000 L $ha^{-1}$ per application falls within the range commonly used in studies evaluating rabbit urine/rabbit-derived liquid biofertilizers on cereal and vegetable crops, where foliar or soil-directed applications between 200 and 1000 L $ha^{-1}$ have produced significant growth and yield responses [17]. The two application dates (at the 2–3 leaf stage and the 6–8 leaf stage) were chosen to target the two physiological windows during which maize is most responsive to readily available nutrient inputs: early vegetative establishment (when root and leaf development set the plant's growth trajectory) and the rapid vegetative growth phase preceding stem elongation, when nitrogen demand sharply increases. This splitting strategy is consistent with reports showing that the timing of biofertilizer application relative to crop growth stage significantly affects its efficacy, with applications at active vegetative stages producing the largest yield gains (e.g., + 59.7% grain yield when a rabbit-manure-derived liquid biofertilizer was applied at the tillering stage in barley [18]).
2. Biochar. The coffee-husk Biochar was applied two weeks before sowing to allow sufficient time for the material to equilibrate with the soil matrix prior to crop establishment. This pre-sowing interval is standard practice in Biochar field trials, as freshly produced Biochar can transiently immobilize plant-available nitrogen through microbial colonization of its porous surface and sorption of soluble nutrients; allowing a short incubation period in situ mitigates this early nutrient-immobilization effect and allows initial pH buffering and cation exchange capacity stabilization to occur before the crop's critical early nutrient-uptake window [12].
3. Mineral fertilizer. The rates and timings used NPK 14-24-14 at 200 kg $ha^{-1}$ applied 15 days after sowing, followed by urea (46% N) at 150 kg $ha^{-1}$ applied 40 days after sowing correspond closely to the official maize fertilization recommendations issued by Cameroon's Ministry of Agriculture and Rural Development (MINADER), which prescribe a basal NPK application near sowing followed by a split urea top-dressing of 100–150 kg $ha^{-1}$ approximately 30–45 days after sowing, timed just before tasseling when nitrogen demand peaks [19]. This two-step regime (phosphorus- and potassium-rich basal dressing followed by a delayed nitrogen top-dressing) is widely used in Cameroonian maize systems and was retained here as the conventional-fertilization control against which the organic and Biochar-based treatments were compared.

The rabbit urine used was alkaline (pH 7.2) and contained 0.34% N, 0.88% P and 1.64% K as major nutrients, together with 0.98% Ca, 0.12% Mg and 0.08% Na as secondary elements. The Biochar is rich in carbon (72%), with moderate levels of potassium (4–6%) and lower amounts of nitrogen (0.8–2%), calcium (0.7–1%), magnesium (0.2–1%), and phosphorus (0.2–0.4%); its alkaline pH ranges from 8 to 10.5, indicating that it may help raise soil pH when applied (Table 1).

Table 1
Chemical characteristics of the rabbit urine and Biochar used in the trial.

| Material | Carbon (%) | Nitrogen (%) | Phosphorus (%) | Potassium (%) | Calcium (%) | Magnesium (%) | Sodium (%) | pH |
|---|---|---|---|---|---|---|---|---|
| Biochar | 72.0 | 0.8–2.0 | 0.2–0.4 | 4.0–6.0 | 0.7–1.0 | 0.2–1.0 | — | 8.0–10.5 |
| Rabbit urine | — | 0.34 | 0.88 | 1.64 | 0.98 | 0.12 | 0.08 | 7.2 |

## 2.3. Experimental design and treatments

A randomized complete block design with three replications was used. The trial area was divided into three blocks, each comprising eight experimental units (24 units in total), each measuring 4 × 3 m (12 $m^2$), separated by 1 m between blocks and 0.5 m between units, for a total trial area of 440 $m^2$. Sowing was carried out at 70 cm between rows and 20 cm between plants within a row, corresponding to a plant density of 71,429 plants $ha^{-1}$ (86 plants per experimental unit). The eight treatments codes and descriptionsare summarized in Table 2.

Table 2
Treatment codes and descriptions.

| Code | Treatments | Dose |
|---|---|---|
| T0 | Unamended control | – |
| T1 | NPK | 200 kg $ha^{-1}$ |
| T2 | Biochar | 2.5 t $ha^{-1}$ |
| T3 | Rabbit urine | 2000 L $ha^{-1}$ |
| T4 | Rabbit urine + NPK | 2000 L $ha^{-1}$ + 200 kg $ha^{-1}$ |
| T5 | Biochar + rabbit urine | 2.5 t $ha^{-1}$ + 2000 L $ha^{-1}$ |
| T6 | Biochar + NPK | 2.5 t $ha^{-1}$ + 200 kg $ha^{-1}$ |
| T7 | Biochar + rabbit urine + NPK | 2.5 t $ha^{-1}$ + 2000 L $ha^{-1}$ + 200 kg $ha^{-1}$ |

## 2.4. Soil sampling and crop management

Composite soil samples (~ 500 g) were collected at 10–20 cm depth using a hand auger prior to trial establishment, and sent to the Ministry of Agriculture, Quality Control laboratory (Yaoundé) for physicochemical analysis. Land preparation included manual ploughing; NPK was band-applied 5 cm from seedlings 15 days after sowing (2.9 g per plant), urea was applied at 40 days after sowing to all treatments except T0, and rabbit urine was applied at the 2–3 and 6–8 leaf stages (1.2 L per experimental unit per application). Biochar was applied two weeks before sowing for corresponding unit.

The experimental soil was classified as clayey according to the USDA texture triangle (51% clay, 32% sand, 17% silt). The soil was strongly acidic ($pH_{water}$ = 5.01), with 5.17% organic matter and a C/N ratio of 17.6. Total nitrogen, total phosphorus and total potassium contents were 0.17%, 7.67% and 1.4%, respectively. The sum of exchangeable bases ($Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$) was 6.54 meq 100 $g^{-1}$, and available phosphorus was very low (6.83 mg $kg^{-1}$), consistent with strong P fixation typical of acidic tropical soils (Table 3).

Table 3
Physicochemical characteristics of the experimental soil before trial establishment.

| Parameters | Unit | Value |
| --- | --- | --- |
| Clay | % | 51 |
| Silt | % | 17 |
| Sand | % | 32 |
| pH (water) | - | 5.01 |
| Organic carbon | % | 3.00 |
| C/N ratio | - | 17.6 |
| Organic matter | % | 5.17 |
| Total nitrogen | % | 0.17 |
| Total phosphorus | % | 7.67 |
| Total potassium | % | 1.4 |
| Calcium | meq/100g | 4.91 |
| Magnesium | meq/100g | 1.50 |
| Sodium | meq/100g | 0.05 |
| Sum of exchangeable bases | meq/100g | 6.54 |
| Available phosphorus | mg/kg | 6.83 |

## 2.5. Data collection

### 2.5.1. Growth variables

Growth data were collected from seven plants selected at the center of each experimental unit to minimize border effects. Growth variables evaluated in this study included leaf number, plant height (cm), leaf area, and collar diameter (cm). Plant height was measured using a graduated ruler, and collar diameter with a tape measure. Leaf number was determined by counting all fully expanded leaves on each plant.

### 2.5.2. Physiological variables

Leaf samples from three plants per unit were collected and analyzed at the Laboratory of Biochemistry and Plant Physiology, Higher Teachers' Training College, University of Yaoundé I. Total soluble sugars were extracted and quantified by the anthrone method (modified from [20]; absorbance at 620 nm against a glucose standard curve). Total soluble proteins were extracted in phosphate buffer (pH 7.2; modified from [21]) and quantified by the Bradford [22] method using Coomassie Brilliant Blue G-250 (absorbance at 595 nm; BSA standard curve). Total phenolic compounds were extracted in 80% methanol (modified from [23]) and quantified with the Folin–Ciocalteu reagent ([24]; absorbance at 760 nm; cinnamic acid standard curve). Total chlorophyll was extracted in 80% acetone and determined spectrophotometrically at 652 nm using the Arnon [25] equation.

### 2.5.3. Soil microbial activity

Overall soil enzymatic (microbial) activity was estimated by fluorescein diacetate (FDA) hydrolysis [26]. Soil samples (2 g) were incubated with sodium phosphate buffer and FDA; the fluorescein released was measured spectrophotometrically at 490 nm, with higher absorbance indicating higher hydrolytic and, by inference, microbial activity.

## 2.6. Statistical analysis

Data were compiled in Microsoft Excel 2019 and analyzed by one-way analysis of variance (ANOVA) using the R software (version 4.3.1) and GenStat 9th Edition. Normality of growth, and physiological data was assessed via the Anderson-Darling test, while homogeneity of variances was evaluated using Levene's test to determine the appropriate statistical approach. Post hoc comparisons of treatment means were performed using Tukey's test at the 5% probability level.

# 3. Results

## 3.1. Effects of fertilizer treatments on maize growth variables

Table 4 shows that maize growth parameters were significantly influenced by the different fertilization treatments. The unamended control (T0) recorded the lowest values for all measured traits, with 11.14 leaves per plant, a collar diameter of 13.90 mm, a plant height of 93.20 cm, and a leaf area of 412.45 $cm^2$. In contrast, all fertilized treatments significantly improved maize growth compared with the control. The combined application of rabbit urine and NPK (T4) produced the greatest collar diameter (27.46 mm) and the tallest plants (176.59 cm), indicating its superior performance for vegetative growth. The biochar + rabbit urine treatment (T5) resulted in the highest leaf number (12.81 leaves $plant^{-1}$), although it was not significantly different from most other fertilized treatments, while the combined application of biochar, rabbit urine, and NPK (T7) produced the largest leaf area (609.91 $cm^2$), remaining statistically comparable to the other fertilized treatments. Sole applications of NPK (T1), biochar (T2), and rabbit urine (T3), as well as the biochar + NPK treatment (T6), also significantly enhanced all growth variables relative to the control, with no significant differences among these fertilized treatments for leaf number, plant height, and leaf area. The rabbit urine–NPK combination (T4) providing the most pronounced improvement in stem development and plant height, whereas the combined application of all three amendments (T7) was particularly effective in increasing leaf area.

Table 4
Effect of treatments on maize growth variables

| Treatments | Leaf number | Collar diameter (mm) | Plant height (cm) | Leaf area (cm²) |
|---|---|---|---|---|
| T0 | 11.14 ± 1.00 b | 13.90 ± 2.19 d | 93.20 ± 6.28 b | 412.45 ± 50.73 b |
| T1 | 12.33 ± 0.64 a | 24.05 ± 0.79 ab | 163.36 ± 13.03 a | 555.73 ± 63.51 a |
| T2 | 12.61 ± 0.58 a | 25.66 ± 1.14 ab | 148.29 ± 16.60 a | 574.64 ± 105.52 a |
| T3 | 12.00 ± 0.29 ab | 19.48 ± 5.46 cd | 147.52 ± 25.57 a | 554.43 ± 23.76 a |
| T4 | 12.76 ± 0.08 a | 27.46 ± 3.98 a | 176.59 ± 13.05 a | 596.36 ± 53.56 a |
| T5 | 12.81 ± 0.58 a | 21.05 ± 3.18 c | 161.71 ± 25.15 a | 603.73 ± 57.36 a |
| T6 | 12.61 ± 0.30 a | 21.86 ± 3.11 bc | 160.81 ± 12.61 a | 576.86 ± 72.94 a |
| T7 | 12.44 ± 0.23 a | 22.00 ± 2.11 bc | 155.51 ± 6.96 a | 609.91 ± 54.89 a |

*a, b, c, d: mean ± SD; means followed by the same letter within a column are not significantly different at 0.05 significant level.*

*T0:Unamended control; T1: NPK (200 kg ha$^{-1}$); T2: Biochar (2.5 t ha$^{-1}$); T3 : Rabbit urine (2000 L ha$^{-1}$) ; T4 : Rabbit urine + NPK (2000 L ha$^{-1}$ + 200 kg ha$^{-1}$); T5 : Biochar + rabbit urine (2.5 t ha$^{-1}$ + 2000 L ha$^{-1}$); T6 : Biochar + NPK (2.5 t ha$^{-1}$ + 200 kg ha$^{-1}$); T7: Biochar + rabbit urine + NPK (2.5 t ha$^{-1}$ + 2000 L ha$^{-1}$ + 200 kg ha$^{-1}$)*

## 3.2. Effects of fertilizer treatments on soil microbial activity

Figure 1 shows that soil microbial activity, assessed by fluorescein diacetate (FDA) hydrolysis, was significantly affected by the different fertilization treatments (Tukey, $P < 0.05$). The combined application of rabbit urine and NPK (T4) produced the highest microbial activity (3.45 µg l$^{-1}$), indicating that this treatment provided the most favorable conditions for microbial metabolism. The sole applications of biochar (T2) and rabbit urine (T3) also markedly enhanced microbial activity compared with the control, although their effects remained significantly lower than that of T4. In contrast, the application of NPK alone (T1) resulted in only a moderate increase in microbial activity (2.40 µg l$^{-1}$), suggesting that mineral fertilization alone was less effective in stimulating soil microbial processes than organic amendments. The combined treatments biochar + rabbit urine (T5) and biochar + NPK (T6) exhibited intermediate microbial activity, with no clear synergistic effect relative to the individual amendments. The unamended control (T0) showed low microbial activity (1.74 µg l$^{-1}$), reflecting the limited biological functioning of the untreated soil. The combined application of biochar, rabbit urine, and NPK (T7) resulted in the lowest microbial activity among all treatments (1.49 µg l$^{-1}$), indicating that the simultaneous incorporation of the three amendments did not promote microbial functioning and may have created less favorable conditions for microbial activity. These results demonstrate that soil microbial activity was strongly dependent on the nature and interaction of the applied amendments, with the rabbit urine–NPK combination emerging as the most effective treatment for enhancing microbial activity under the conditions of this study.

## 3.3. Effects of fertilizer treatments on maize physiological variables

Figure 2 illustrates that the physiological characteristics of maize responded differently to the fertilization treatments, with significant variations observed for all measured variables (Tukey, $P < 0.05$). Total phenolic content (Fig. 2A) was greatest in plants receiving NPK alone (T1), rabbit urine alone (T3), and the combined application of rabbit urine and NPK (T4), with no significant differences among these treatments. In contrast, biochar alone (T2) resulted in lower phenolic accumulation (9.99 µg eq $g^{-1}$ FW), while the biochar + NPK treatment (T6) recorded the lowest value (9.17 µg eq $g^{-1}$ FW). The biochar + rabbit urine (T5) and the combined application of biochar, rabbit urine, and NPK (T7) produced intermediate phenolic levels.

The treatments also significantly influenced soluble protein content (Fig. 2B). The highest protein concentration (48.58 µg BSA-eq $g^{-1}$ FW) was obtained with the combined application of biochar and rabbit urine (T5), followed by the rabbit urine + NPK treatment (T4) (47.01 µg BSA-eq $g^{-1}$ FW), with no significant difference between them. Rabbit urine applied alone (T3) resulted in the lowest protein content, similar to the unamended control (T0). The remaining treatments, including NPK alone (T1), biochar alone (T2), biochar + NPK (T6), and the triple combination (T7), produced intermediate values.

Total chlorophyll content (Fig. 2C) exhibited a distinct response to fertilization. The highest chlorophyll concentration (7.02 µg $mL^{-1}$), was recorded in the biochar + NPK treatment (T6), which was significantly higher than all other treatments. By contrast, plants fertilized with NPK alone (T1) had the lowest chlorophyll content (1.81 µg $mL^{-1}$). The other treatments displayed intermediate values, indicating that combining organic and mineral amendments affected chlorophyll accumulation differently depending on the type of amendment applied.

A similar treatment effect was observed for soluble sugars (Fig. 2D). Plants receiving NPK alone (T1) accumulated the highest soluble sugar content (5.44 mg $mL^{-1}$ $g^{-1}$ FW), followed by those treated with biochar + NPK (T6) (5.29 mg $mL^{-1}$ $g^{-1}$ FW), although these two treatments did not differ significantly. Intermediate sugar concentrations were measured in the control (T0), biochar (T2), rabbit urine (T3), and biochar + rabbit urine (T5) treatments. In contrast, the lowest concentrations were observed when rabbit urine was combined with mineral fertilizer (T4) or with both Biochar and NPK (T7), each recording 4.36 mg $mL^{-1}$ $g^{-1}$ FW. Overall, the physiological performance of maize varied according to the fertilization strategy, with each treatment preferentially enhancing specific physiological traits rather than consistently improving all variables simultaneously.

## 4. Discussion

The present study aimed to evaluate the effect of an organic amendment based on biochar combined with rabbit urine on the agronomic and physiological performance of maize and on soil microbiological activity. Growth variables were improved by fertilization relative to the unamended control. The largest collar diameter and plant height were recorded under the rabbit urine + NPK combination (T4), likely reflecting a nutritional complementarity between a readily mineralizable organic nitrogen source and a balanced mineral supply of N, P and K, in agreement with Achiri et al. [27], who reported that balanced N-P-K fertilization significantly improves vegetative growth and stem diameter in maize in the North-West Region of Cameroon. The markedly lower performance of the unadamended control aligns with reports that the absence of fertilizer input strongly limits vegetative tissue growth in maize.

Leaf number was maximized under the Biochar + rabbit urine combination (T5), consistent with the reported capacity of Biochar to raise soil pH and improve nutrient retention in acidic soils, thereby complementing the readily available nutrients supplied by rabbit urine and jointly supporting new leaf formation [28]. Leaf area followed a similar pattern, being highest under the fully combined Biochar + rabbit urine + NPK treatment (T7), which may reflect the additive benefit of Biochar-driven amelioration of soil acidity combined with the complementary nutrient supply from NPK and rabbit urine [28, 27]. Biochar's liming action is driven by the release of hydroxyl ions and basic cations from its ash fraction, which neutralize soil $H^+$ and reduce the exchangeable and soluble fractions of $Al^{3+}$ and $Mn^{2+}$, while its high specific surface area and density of oxygen-containing functional groups increase cation exchange capacity and retain the nutrients supplied by rabbit urine against leaching, thereby sustaining the nutrient supply needed for successive leaf initiation [29].

Soil microbial activity, as inferred from FDA hydrolysis, was highest under the rabbit urine + NPK treatment (T4), suggesting a synergy between an easily mineralizable organic input and directly available mineral nutrients that jointly favor microbial community development, consistent with previous findings that combined organic-mineral fertilization stimulates enzymatic activity and microbial biomass more than unbalanced fertilization regimes [30]. This pattern is consistent with reports that carbon-rich organic amendments can, on their own, restore microbial biomass and stimulate hydrolytic enzyme activity in nutrient-poor tropical soils, an effect that is further reinforced when a readily available mineral nutrient source is supplied alongside the organic input to relieve the nutrient limitation of the microbial community [31]. Notably, the fully combined treatment (T7) recorded the lowest microbial activity of all treatments, even below the control, a counter-intuitive result that may reflect an excess or imbalance of inputs (e.g., localized changes in soil pH, ionic strength, or substrate competition) under the triple combination; this warrants further investigation, ideally including direct microbial biomass and community composition assays, since FDA hydrolysis is an indirect, broad-spectrum proxy for microbial activity rather than a measure of microbial abundance or diversity. This decline is also consistent with evidence that mineral nitrogen inputs, whatever their chemical form, can directly suppress soil microbial respiration once soil nitrogen availability rises above the level required by the microbial community, an inhibitory effect attributed to a shift in microbial metabolic strategy away from nitrogen-mining activity rather than to changes in soil pH [32], and which may help explain why microbial activity under the triple combination (T7) fell even below that of the unamended control. This apparent inhibition under the triple combination is consistent with recent evidence that, although moderate co-application of organic and mineral inputs stimulates microbial biomass and enzyme activity, once inputs exceed the assimilative capacity of the microbial community, excessive and unevenly distributed nutrient and salt loads reduce microbial carbon-use efficiency and destabilize enzyme-producing microbial networks, so that biological activity can fall below that of unamended soil despite higher nutrient availability [33, 34].

Physiological responses varied by variable and treatment, reflecting distinct underlying processes. Phenolic compounds, secondary metabolites involved in plant defense against environmental stress, were highest under rabbit urine alone (T3), possibly because the readily assimilable nutrients in rabbit urine stimulate metabolism and redirect part of the carbon pool toward phenolic biosynthesis, consistent with reports that liquid organic fertilizers derived from livestock waste increase polyphenol content in leafy vegetables such as Chinese cabbage [35]. Conversely, the lowest phenolic content, recorded under Biochar + NPK (T6), may indicate a comparatively lower metabolic stress status under this combination. This response is consistent with the operation of the phenylpropanoid pathway, in which phenylalanine ammonia-lyase (PAL) diverts carbon from primary metabolism toward the synthesis of phenolic acids and flavonoids whenever plants perceive a nutritional or osmotic imbalance, so that the fast, unbuffered nutrient pulse delivered by rabbit urine alone may transiently upregulate PAL activity and phenolic accumulation more than treatments in which Biochar moderates nutrient release [36].

Soluble protein content, an indicator of plant nitrogen status, was maximized under Biochar + rabbit urine (T5), which may reflect a complementary action whereby Biochar improves soil structure and water/nutrient retention, enhancing root uptake of the nitrogen supplied by rabbit urine and thereby promoting foliar protein synthesis, in line with reports that organic amendments enhance maize protein content through improved nutrient availability and root absorption efficiency [37, 38]. This agrees with the fact that nitrogen taken up by the root, whether as nitrate or ammonium, must first be reduced and incorporated into amino acids via nitrate reductase and the glutamine synthetase/glutamate synthase (GS-GOGAT) cycle before being used for protein synthesis, and Biochar's improvement of soil aggregation, water retention and cation exchange capacity is reported to prolong nitrogen availability to roots and support sustained GS-GOGAT activity, which would explain the higher soluble protein accumulation observed when Biochar was combined with rabbit urine [39].

Total chlorophyll content, closely tied to nitrogen and magnesium availability and photosynthetic capacity, was highest under Biochar + NPK (T6), possibly reflecting a complementary mechanism whereby NPK supplies nitrogen and potassium directly while Biochar improves the physicochemical conditions that facilitate their uptake and use in chlorophyll biosynthesis, consistent with reports that combining organic amendments with mineral fertilization improves chlorophyll content by increasing the availability of essential minerals [37]. This supports the central biochemical role of magnesium, which forms the coordinating metal of the chlorin ring of chlorophyll and is also an essential cofactor for magnesium chelatase, RuBisCO activation and glutamine synthetase, so that treatments best able to sustain both nitrogen supply and magnesium/base-cation availability, such as the combination of Biochar's cation exchange capacity with NPK's direct N and K input, would be expected to maximize chlorophyll biosynthesis and photosynthetic capacity [40].

Soluble sugar content, reflecting direct photosynthetic output, was highest under NPK alone (T1), likely due to the immediate availability of mineral nitrogen, which rapidly stimulates chlorophyll synthesis and photosynthetic activity, whereas organic amendments release nutrients more gradually and may therefore temporarily limit photosynthetic intensity and sugar accumulation, in agreement with reports that adequate nitrogen nutrition promotes soluble carbohydrate accumulation in maize through enhanced photosynthesis and carbon metabolism [41]. The immediate availability of mineral nitrogen from NPK rapidly activates nitrate reductase and downstream carbon nitrogen metabolic enzymes, favoring the accumulation of non-structural carbohydrates in leaves, whereas the more gradual, microbially mediated mineralization of nitrogen from organic amendments such as rabbit urine and Biochar delays this stimulation and channels a larger share of assimilated carbon toward amino acid and protein synthesis rather than transient soluble-sugar accumulation [42].

## 5. Conclusion

This study evaluated the effects of an organic amendment based on Biochar enriched with rabbit urine, alone or combined with mineral NPK fertilizer, on the agro-physiological performance of maize and on soil microbial activity in Bafia, Cameroon. All fertilized treatments significantly outperformed the unamended control across growth, physiological and microbiological variables. The combination of rabbit urine and NPK (T4) was the most effective at stimulating soil microbial activity and vegetative growth parameters, while treatments combining Biochar with rabbit urine or with NPK were distinguished, respectively, by the highest foliar soluble protein and total chlorophyll contents, reflecting a more favorable nutritional and photosynthetic status. These findings indicate that the combined use of Biochar, rabbit urine and NPK can improve both the agro-physiological performance of maize and soil microbial activity, offering a productive and environmentally sound fertilization option. Integrating these organic-amendments into maize cropping systems in tropical, acidic-soil regions appears to be a judicious strategy for sustainable soil fertility management and improved food security.

## Declarations

# Competing interests

The authors declare no competing interests.

# Ethics, Consent to participate, and Consent to publish declarations

The collection of plant material (maize leaves, *Zea mays*) used in this study complied with applicable local and national guidelines.

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# Author Contribution

Suzanne Olive Endong Assougouma, Martial Hugues Kompané and Beaulys Fotso conducted the field experiment, collected and analyzed the data, and drafted the manuscript. Hervé kuietche Mube and Beaulys Fotso designed and supervised the study, provided scientific guidance, and revised the manuscript. All authors read and approved the final manuscript.

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# Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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# Figures

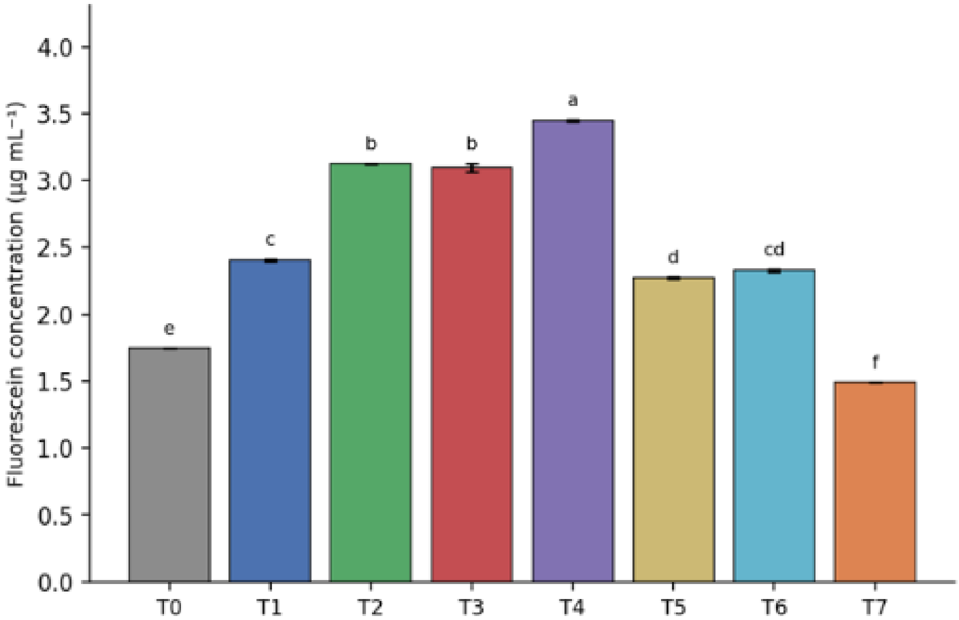


**Figure 1**

Effect of treatments on soil microbial activity (FDA hydrolysis).

*Error bars = SD; bars sharing a letter are not significantly different (Tukey, $P < 0.05$). T0:Unamended control; T1: NPK (200 kg $ha^{-1}$); T2: Biochar (2.5 t $ha^{-1}$); T3 : Rabbit urine (2000 L $ha^{-1}$) ; T4 : Rabbit urine + NPK (2000 L $ha^{-1}$ + 200 kg $ha^{-1}$); T5 : Biochar + rabbit urine (2.5 t $ha^{-1}$ + 2000 L $ha^{-1}$); T6 : Biochar + NPK (2.5 t $ha^{-1}$ + 200 kg $ha^{-1}$); T7: Biochar + rabbit urine + NPK (2.5 t $ha^{-1}$ + 2000 L $ha^{-1}$ + 200 kg $ha^{-1}$)*

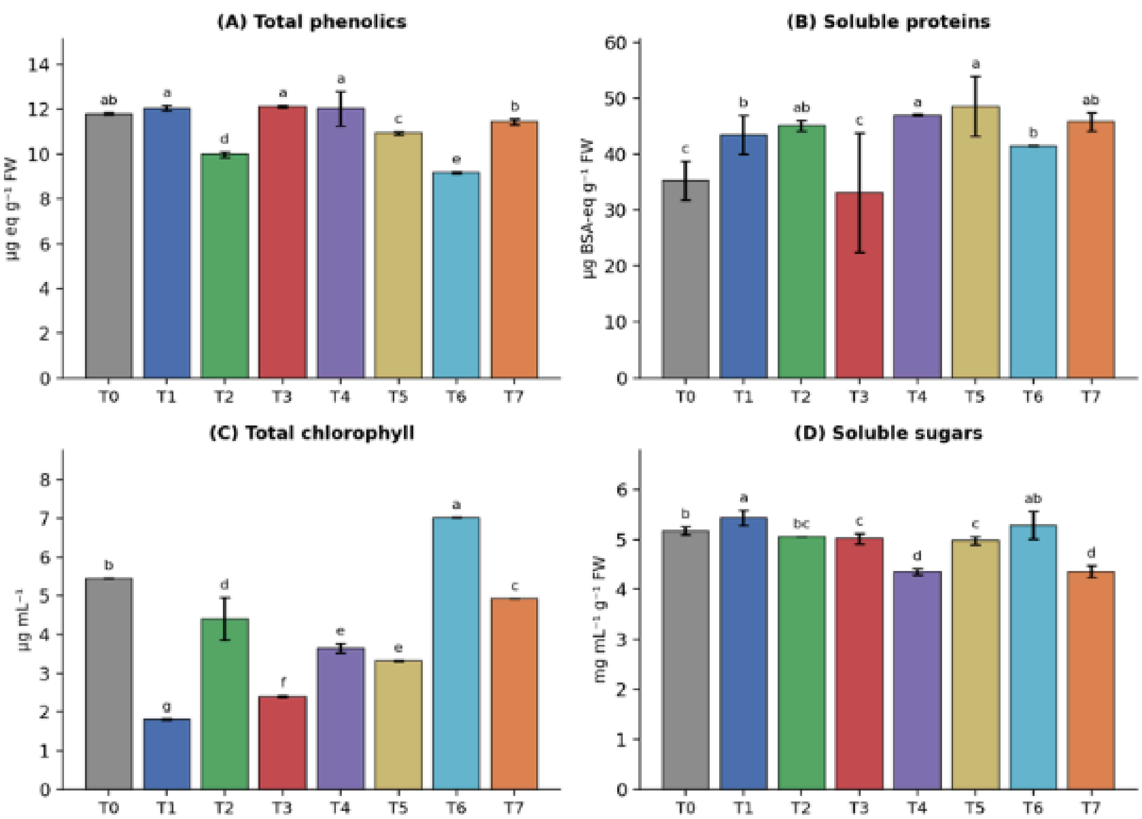

**Figure 2**

Effect of Biochar, rabbit urine and NPK, alone or combined, on maize physiological variables: (A) total phenolics, (B) soluble proteins, (C) total chlorophyll, (D) soluble sugars.

*Error bars = SD; bars sharing a letter are not significantly different (Tukey, $P < 0.05$). T0:Unamended control; T1: NPK (200 kg $ha^{-1}$); T2: Biochar (2.5 t $ha^{-1}$); T3 : Rabbit urine (2000 L $ha^{-1}$) ; T4 : Rabbit urine + NPK (2000 L $ha^{-1}$ + 200 kg $ha^{-1}$); T5 : Biochar + rabbit urine (2.5 t $ha^{-1}$ + 2000 L $ha^{-1}$); T6 : Biochar + NPK (2.5 t $ha^{-1}$ + 200 kg $ha^{-1}$); T7: Biochar + rabbit urine + NPK (2.5 t $ha^{-1}$ + 2000 L $ha^{-1}$ + 200 kg $ha^{-1}$)*