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Effect of non-conventional organic fertilizer (horn and hoof meal) on the development of cacao (*Theobroma cacao* L.)

Efecto del abono orgánico no convencional (harina de cuernos y pezuña) en el desarrollo de cacao (*Theobroma cacao* L.)

Zumaeta-Barbarán, Rodrigo S.[1*]
Arévalo-Hernández, Cesar O.[2,3]

[1]Universidad Científica del Sur. Lima, Perú
[2]Instituto de Cultivos Tropicales. Tarapoto, Perú
[3]Universidad Nacional Autónoma de Alto Amazonas. Yurimaguas, Perú

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Autor de correspondencia*: rszumaetab@gmail.com
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ABSTRACT

Cacao is a vital cash crop in cacao-producing countries, often grown with inadequate management. This study aimed to evaluate the impact of horn and hoof meal (HHM) on the development of seven-year-old cacao hybrids. Conducted at the Tropical Crops Institute in San Martin, Peru, (2018-2020), the experiment design was a CRD with four replicates. The treatments included: 332.5 g tree^{-1} (T1), 249.9 g tree^{-1} (T2), 166.6 g tree^{-1} (T3), 83.3 g tree^{-1} (T4), 72.5 g tree^{-1} (T5), and 0 g tree^{-1} (T0), with additional triple superphosphate and potassium chloride per tree. Results showed that HHM and urea had similar effects on leaf area, however, HHM significantly improved SPAD content and dry biomass of lateral roots. The optimal doses were 83.3 g tree^{-1} (T4) for leaf area, 332.5 g tree^{-1} (T1) for SPAD content, and 83.3 g tree^{-1} (T4) for dry biomass of lateral roots. This suggests that HHM can be an effective alternative to urea for cacao fertilization, particularly in improving SPAD content and root biomass.

Keywords: leaf area; biomass; fertilization; nitrogen; byproduct management

RESUMEN

El cacao es un cultivo comercial de importancia mundial, frecuentemente manejado de manera inadecuada. Este estudio evaluó el efecto de la harina de cuerno y pezuña (HCP) sobre el desarrollo de híbridos de cacao de 7 años. El experimento, fue realizado en el Instituto de Cultivos Tropicales en San Martín, Perú (2018-2020). Se utilizo un DCA, con cuatro repeticiones. Los tratamientos consistieron en 332,5 g árbol^{-1} (T1), 249,9 g árbol^{-1} (T2), 166,6 g árbol^{-1} (T3), 83,3 g árbol^{-1} (T4), 72,5 g árbol^{-1} (T5), y 0 g árbol^{-1} (T0), con superfosfato triple y cloruro potásico adicionales por árbol. Se midieron la biomasa seca de las raíces laterales, el contenido de SPAD y el área foliar. Los resultados indicaron que el HCP y la urea tuvieron efectos similares en el área foliar, pero el HCP mejoró significativamente el contenido de SPAD y la biomasa de las raíces laterales. Se identificaron como dosis óptimas 83,3 g árbol^{-1} (T4) para área foliar, 332,5 g árbol^{-1} (T1) para contenido de SPAD, y 83,3 g árbol^{-1} (T4) para la biomasa de raíces.

Palabras clave: área foliar; biomasa; fertilización; nitrógeno; gestión de subproductos

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1. INTRODUCTION

Cacao (*Theobroma cacao* L.) is a perennial dicot crop belonging to the Malvaceae family (Zhang & Motilal, 2016). Its domestication and use date back approximately 5,000 years ago, as evidenced by findings in Palanda, Ecuador and Jaen,Perú (Lamber et al., 2020; Bustamante et al.,2022). Although its early utilization dates back millennia, large-scale global demand for cacao only emerged in the 19th century, notably in Colonial Africa (Ross, 2014). Throughout history, cacao beans have played a critical role in cultural practices and economic development (Walker, 2000; Zarillo et al., 2018).

At present, cacao production sustains the livelihoods of nearly six million smallholder farmers worldwide (Niether et al., 2019; Bustamante et al., 2022) who benefit from the pharmaceutical, cosmetic and chocolate industries (Franco et al., 2013; Higginbotham & Taub, 2015; Bustamante et al., 2022), and recently, from the carbon market (Borden et al., 2019). Nevertheless, cacao is predominantly produced in low-input systems despite the fact that this tree demands substantial amounts of macronutrients and micronutrients from the soil in order to achieve high yields (Zhang & Motilal, 2016; Lamber et al., 2020; Goudsmit et al., 2023). Among all nutrients, Nitrogen (N) is the most demanded and responsible of yield in cacao (van Vliet & Giller, 2017; Marrocos et al., 2020; Quintino R. de et al.,2020).

For instance, the amount of this nutrient removed by 1000 kg of dry cacao beans varies from 19.2 kg to 39.3 kg for beans and 10.6 kg to 31.4 kg for husks (Hartemink, 2005). Quintino R. de et al. (2020) found that nitrogen is directly associated with cacao crops that have high and very high biomass of cacao beans. They also noted that N concentration in dry cacao beans was not correlated with other nutrients, indicating its unique role in plant development.

Similarly, Marrocos et al. (2020) reported that medium, high, and very high productivity in cacao plantations correspond to foliar N concentrations ranging from 22 to 25 g/kg. Additionally, nitrogen stimulates leaf flushing, which increases leaf area and canopy formation, thereby contributing to yield (van Vliet & Giller, 2017). Cacao primarily takes up nitrogen through its lateral roots, which are predominantly found within top 30 cm of the soil (Kummerow et al., 1982; Zaia et al., 2012; van Vliet & Giller, 2017). This is significant because in tropical regions, the major part of nutrients is found in the top 25 cm of soil (Hartemink, 2005).

Nitrogen also functions as a key structural component of chlorophyll; approximately 75% of leaf nitrogen is directed to the chloroplast (Romero et al., 2022). This is relevant because higher chlorophyll concentration is associated with higher photosynthetic rates, thereby enhancing plant growth and development through greater energy availability for processes such as carbon fixation and biomass production (Foyer et al., 2017; Flexas & Carriquí, 2020; Muhie, 2022).

Several experiments have been carried out to assess the effect of organic fertilizers on the nutrition and productivity of crops such as cereals, vegetables, fruits, root and tuber crops, with a focus on maintaining soil fertility and productivity (de Ponti et al., 2012; Aguirre Yato & Alegre Orihuela, 2015; van Vliet & Giller, 2017; Lamber et al., 2020; Arum et al., 2023).

According to Arum et al. (2023) the application of an organic liquid fertilizer (13-5-14) on cacao seedlings grown in a medium of cow manure significantly improved the stem diameter, leaf length, leaf width, root length and both fresh and dry weights of the seedlings. Lambert et al. (2020) found that the combination of: NPK dolomite, rock phosphate and an organic fertilizer (comprising cow manure, chicken manure, sugarcane stem waste and oil palm bunch waste) improved cacao growth and establishment, as well as soil properties.

The use of animal-by products as a soil amendment or as an input for compost has been shown to positively affect plant growth and development (Cayuela et al., 2009; Aguirre Yato & Alegre Orihuela, 2015).

Additionally, it enhances soil quality (Juknevičienė et al., 2019; van der Sloot et al., 2022) and reduces the emission of greenhouse gases when properly managed (Cayuela et al., 2010; Goldan et al., 2023).

For example, Juknevičienė et al. (2019) observed that the application of horn-manure significantly increased the soil phosphorus, potassium and nitrogen levels and also enhanced the soil enzyme activity, particularly urease and saccharase activities. Similarly, Žibutis et al. (2013) noted that the application of horn shaving and horn core to a winter wheat field increased the nitrogenous compounds in the soil and resulted in higher crop yields, demonstrating their effectiveness in enhancing plant nutrient uptake, especially during warm and humid weather conditions. Furthermore, van der Sloot et al. (2022) found that applying organic amendments made by herbaceous road verge cuttings mixed with cow slurry and with a C:N ratio of 10 increased crop biomass of spring wheat and a decreased mineral soil N content.

Another animal by-product utilized in agriculture is horn and hoof meal (HHM), a non-conventional organic fertilizer, known for its slow-release properties due to sulphur-containing amino acids such as methionine, cystine and cysteine which contribute to its chemical structure (Cayuela et al., 2009). The content of nitrogen (N) ranges from 13.58 % (Aguirre Yato & Alegre Orihuela, 2015) to 17% (Cayuela et al., 2010) and the availability of it can vary based on the method of obtention and particle size, with finer particles exhibiting higher availability for plant uptake (Owen et al., 1953). For the obtention, Owen et al. (1953) suggest to submit HHM to an autoclave for an hour with a pressure of 15 psi. In addition, processing methods that retain amino acids present in HHM can improve the quality in terms of nutrients composition for agricultural and composting purposes (Cayuela et al., 2009).

There is limited research on the effect of non-conventional organic fertilizers, such as HHM, on cacao growth. Due to its nitrogen content and the positive impact on soil-plant-microbe system as well, HHM could enhance cacao development and increase yields without depleting the soil. Therefore, the aim of this study was to determinate the effect of the horn and hoof meal (HHM) on dry biomass of lateral roots, SPAD content and leaf area in cacao grown in tropical conditions in San Martín, Perú.

2. MATERIALS Y MHETODS

2.1. Experimental site

A field experiment was conducted at the “Juan Bernito” station of the Tropical Crops Institute (San Martín, Perú) (Figure 1) between December 2018 and March 2020. During this period, the maximum recorded temperature was 36.2 ° C in September 2019 and the minimum was 15.8 ° C in August 2019. The highest monthly cumulative rainfall value observed was 278 mm for January 2019, while the lowest was 38.5 mm in June 2019 (National Meteorological and Hydrological Service of Peru, 2020) (SENAMHI, 2020). The soil is classified as an acidic Ultisol with variable texture and a depth greater than 3 m.

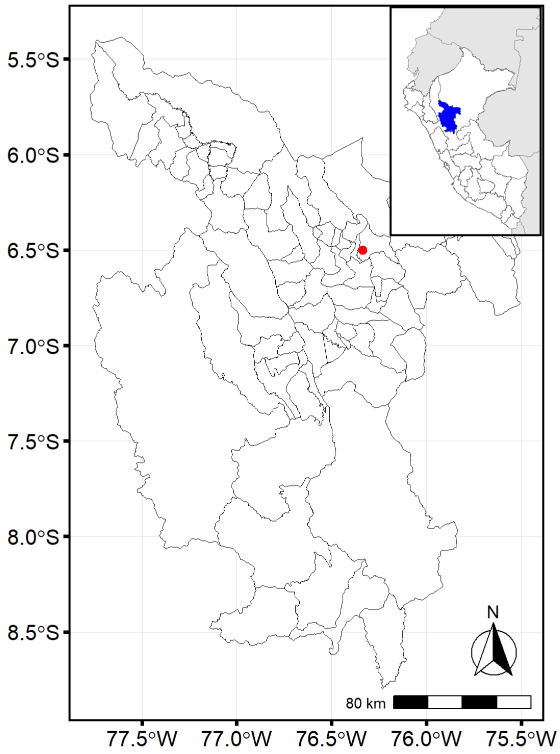

Figure 1. Location of San Martín, Perú (blue polygon) and ubication of "Juan Bernito" station (red dot) from the Tropical Crop Institute (ICT), San Martín, Perú

2.1. Horn and hoof meal

150 kg of horn and hoof meal was purchased from a local producer named Antonio Documet. A significant portion of this organic fertilizer was analyzed in a private laboratory (Table 1). The nitrogen percentage of HHM used in this study was 15%, which was higher than the reported by Aguirre Yato & Alegre Orihuela (2015) (13.5) and lower than Cayuela et al. (2010) (17 %). The pH value of HHM was 5.93, categorizing it as slightly acidic.

Table 1. Chemical Analyze of horn and hoof meal used in the trial

Chemical Parameters	Values	Units
pH (extract 1/10)	5.93	-
Sulfur	2.32	%
Boron	< 5.00	mg/kg
Calcium	0.426	%
Chlorides	1050	mg/kg
Copper	6.50	mg/kg
Phosphorus	0.08	%
Iron	6091	mg/kg
Magnesium	< 0.06	%
Manganese	66.4	mg/kg
Total Organic Matter	94.9	%
Molybdenum	< 2.50	mg/kg
Total Nitrogen	15.0	%
Potassium	0.149	%
C/N Ratio	3.66	
Sodium	926	mg/kg
Zinc	232	mg/kg
Electrical conductivity (extract 1/10)	2696	µS/cm a 20º C
Nitrates	< 0.06	%
Ammonium	> 1000	mg/kg

2.2. Experimental design

The experiment utilized a completely randomized design (CRD) with nitrogen fertilization as the fixed factor (Figure 2). This factor consisted of six levels spanning six treatments: treatment "0" served as the control (0 kg N ha^{-1}), treatments "1" to "4" represented different doses of HHM (200, 150, 100 and 50 kg N ha^{-1}) and treatment "5" (150 kg N ha^{-1}) used a commercial formulation with urea as the principal source of nitrogen, each treatment had four replicates.

The respective doses per tree (g tree^{-1}) were as follow: T0 (0 N, 47.5 P_2O_5 and 32.5 K_2O), T1 (332.5 N, 47.5 P_2O_5 and 32.5 K_2O), T2 (249.9 N, 47.5 P_2O_5 and 32.5 K_2O), T3 (166.6 N, 47.5 P_2O_5 and 32.5 K_2O), T4 (83.3 N, 47.5 P_2O_5 and 32.5 K_2O), and T5 (72.5 N, 47.5 P_2O_5 and 32.5 K_2O). These treatments were applied to 24 seven-year-old cacao trees, previously pruned, in January, March, July and October of 2019. For fertilizer application, first the leaf litter was moved aside, then the fertilizers were placed around the cacao trunk at a distance of 1m and finally the leaflitter was returned to in its initial position (Niether et al., 2019).

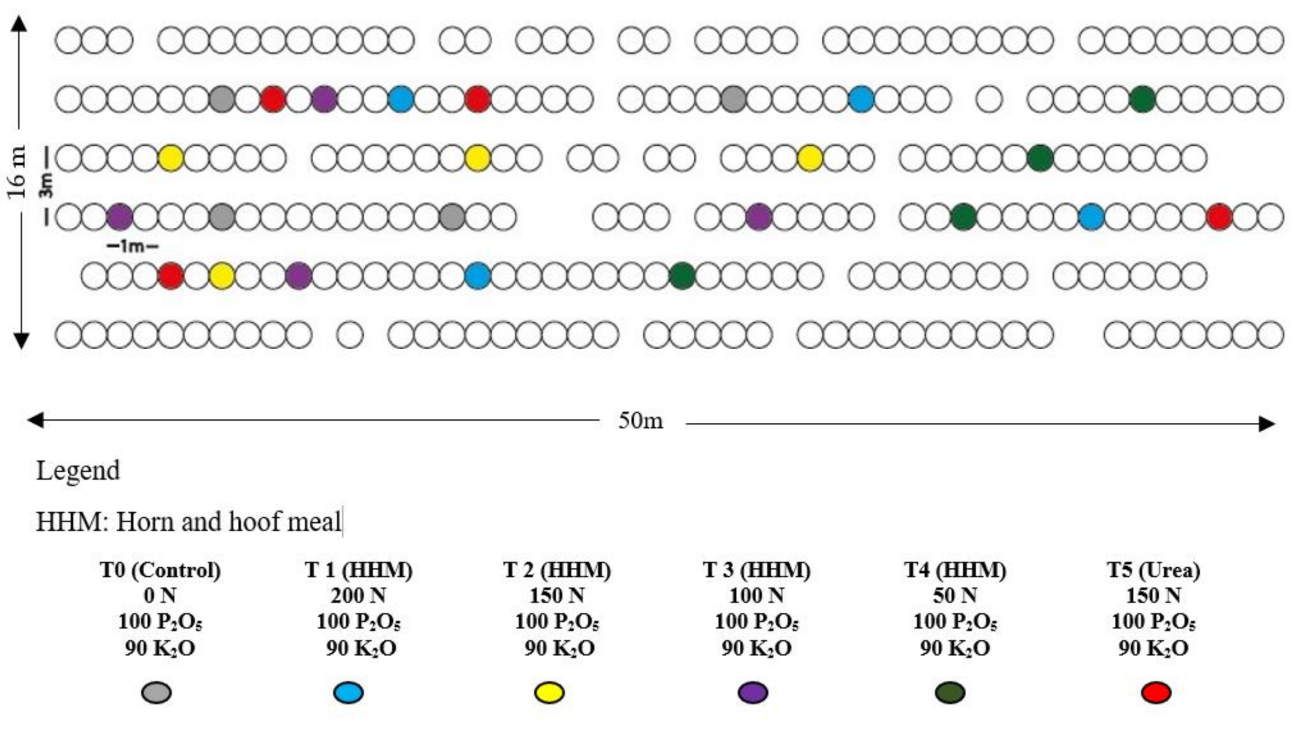

Figure 2. Distribution of each treatment in the experimental site located in the "Juan Bernito" station from the Tropical Crops Institute (ICT), San Martín, Perú

2.3. Biometrics Indicators

2.3.1. Dry biomass of lateral roots

Five rings, each 2.76 cm in height and 3 cm in diameter, were positioned 50 cm from the cacao trunk. The spacing between these was 40 cm (Niether et al., 2019) with the exception of one, which was 25 cm from the edges. Once positioned, they were buried in the top 10 cm of the soil and then retrieved with the spade. The samples were situated in trays, one per tree, and subsequently transported to the laboratory.

Any material or colloids that did not belong to the lateral roots were eliminated with the assistance of tweezers and a mesh strainer. The moist roots were then weighed and placed in a "Memmert" oven, at 75° C for 3 days. To ensure thorough drying efficiency, some roots were fractured to confirm dryness; finally, the dried roots were weighed (Schuurman & Goedewaagen, 2010). Measurements were taken in July 2019 and March 2020.

2.3.2. Soil Plant Analysis Development (SPAD)

The values from this indicator were obtained by measuring the fourth fully expanded leaf from the top of the branch with the portable SPAD meter "Konica Minolta". These readings were taken at two-thirds of the distance from the leaf base (Yuan et al., 2016). Four leaves were evaluated per experimental unit and the considerations were similar to those for leaf area. Measurements were taken in July 2019 and January 2020.

2.3.3. Leaf area

To asses this metric, the canopy of each tree was initially segmented into four sectors based on the cardinal directions. Subsequently, 10 leaves per tree were evaluated using the portable leaf meter LAM-B, taking into account the lower and upper regions of the canopy, while preventing direct exposure to sunlight on the portable device. This procedure was conducted with the subsequent considerations: i) examining heathy leaves and ii) omitting newly formed and extremely mature leaves (Suárez Salazar et al., 2018). Measurements were taken in July 2019 and March 2020.

2.4. Statistical analysis

An analysis of variance (ANOVA) and the Scott-Knott test (Francisco & Carlos, 2016; Malaquias et al., 2023) were conducted at a 95% confidence level to detect significant differences and identify homogeneous subsets among the treatments. Additionally, a linear regression model was constructed to explain SPAD content in relation to nitrogen doses. Assumptions of homoscedasticity and normal distribution of residuals were assessed using the Levene and Shapiro-Wilk tests, respectively. All statistical analyses were performed using RStudio version 4.2.2. (Goudsmit et al., 2023).

3. RESULTS and DISCUSSION

3.1. Soil characteristics of the experimental site

The initial (December 2018) chemical and physical properties of the surface soil were as follows: pH of 6.30, electrical conductivity (EC) of 0.31 ds/m, $CaCO_3$ less than 0.3 %, 1.26% of organic matter (O.M), 0.06 of nitrogen (N), 7.96 ppm of phosphorus (P) and 22 ppm of potassium (K). In terms of exchangeable cations for Ca^{2+}, Mg^{2+}, K^+, Na^+ and Al^{3+} + H^+ the values were: 9.79 cmol⁺/kg, 6.23 cmol⁺/kg, 0.40 cmol⁺/kg, 0.06 cmol⁺/kg, 0.10 cmol⁺/kg and 0.00 cmol⁺/kg, respectively. According to the mechanical analysis, the soil had 61.52 % of sand, 3.28 % of silt and 35.20% of clay which correspond to texture class of sandy clay.

These values suggest that the experimental site possesses, partially, ideal soil conditions for the growth of cacao. Specifically, chemical indicators such as i) acidity level and ii) electrical conductivity (EC), exhibit favorable values for the growth of this vegetation; nevertheless, there are low levels of organic matter (OM), N, P and K as well. In terms of physical attributes, the soil texture is classified as clay-sandy with the proportions of each colloid falling within the optimal interval (Hartemink, 2015; van Vliet & Giller, 2017; Asigbaase et al., 2021).

3.2. Dry biomass of lateral roots

Significant differences were observed among the HHM, commercial, and control treatments in both July 2019 (P = 0.0006077) (Figure 3) and March 2020 (P = 2.004×10-5) (Figure 3). In July, the highest mean was observed in T3, with a value of 5.12 mg/cm^3, followed by T1 (4.44 mg/cm^3), T4 (3.75 mg/cm^3), T5 (3.67 mg/cm^3), T0 (3.46 mg/cm^3), and T2 (3.10 mg/cm^3). Subsequently, subsets were established based on the Scott-Knott test at P < 0.05: subset "a" (T3 and T1) and subset "b" (T4, T5, T0, and T2) (P = 0.0033).

However, in March (Figure 3), there were no significant differences (P = 0.24100) among the HHM doses, which were: 6.40 mg/cm^3 (T4), 5.96 mg/cm^3 (T1), 5.83 mg/cm^3 (T3), and 5.48 mg/cm^3 (T2). These values represented increases of 71%, 34%, 14%, and 77%, respectively, compared to July (Figure 3). For T0 and T5, the mean values were 4.17 mg/cm^3 and 3.83 mg/cm^3, indicating increases of 20% and 4%, respectively.

Consequently, the subsets for March 2020, according to the Scott-Knott test at $P < 0.05$, were: subset "a" (T4, T1, T3, T2) and subset "b" (T0 and T5) ($P = 0.00035$).

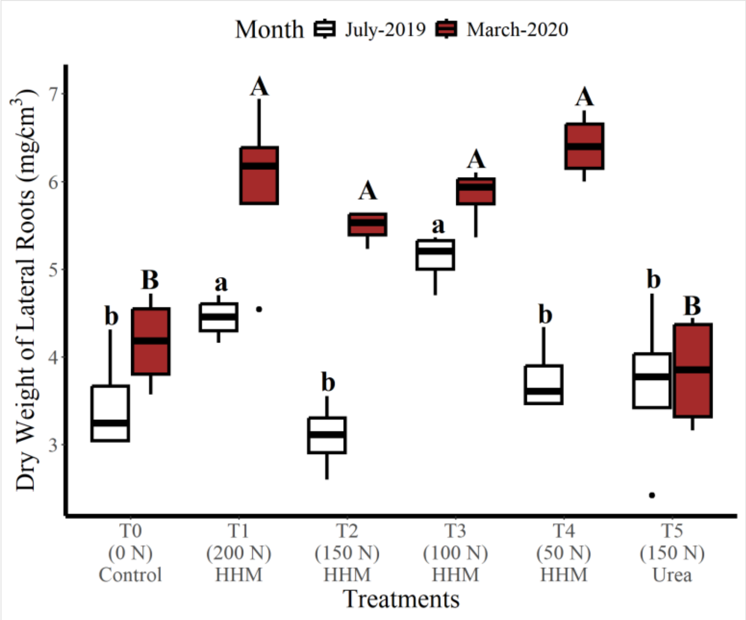

Figure 3. Dry biomass of lateral roots (mg/cm3) values of *Theobroma cacao* L measured in July 2019 and March 2020. Data represent averages (n = 4) and error bars indicate standard error. Bars labeled with different letter within the same month are significantly different among treatments, as determined by Scott-Knott test at P < 0.05

These findings contrast with those of Arthur et al. (2019), who reported minimal dry matter production of roots (g $plant^{-1}$) in cacao seedlings grown in a medium of soil sample from an old cacao plantation with an NPK content of 0.04%, 12.80 mg kg^{-1}, and 17.2 mg kg^{-1}, respectively, when fertilized with green-grown compost containing 0.01% N, 5380.70 mg kg^{-1} P, and 17.2 mg kg^{-1} K. In contrast, Arum et al. (2023) observed that the significant interaction between a growing medium of chicken manure (1.00% N, 0.80% P, 0.39% K, and a C/N ratio of 21.8) and the application of 150 ml $polybag^{-1}$ of organic liquid fertilizer (12.98% N, 5.12% P, 14.20% K) resulted in the highest root length (cm) in cacao seedlings. For root number of cacao seedlings, the highest values were obtained in a medium of cow manure (0.57% N, 0.23% P, 0.60% K, and a C/N ratio of 33.9) and fertilized with 50 ml $polybag^{-1}$ of the organic liquid fertilizer.

These outcomes are consistent with Niether et al. (2019) who noted that total lateral root production in cacao was four times greater in organic monoculture system fertilized once a year with compost (24–17–20–18 kg N_{total}-P2O5-K2O-MgO ha^{-1}) compared to conventional monoculture system fertilized with synthetic product (18–12–24–4kg N_{total}-P2O5-K2O-MgO ha^{-1}). This evidence suggests that organic fertilizers, when integrated into the agricultural system with consideration of their chemical composition, the specific attributes of the soil or growing medium, as well as the developmental stage of the plant, significantly promote the growth of cacao roots, thereby facilitating an efficient uptake of nutrients.

The benefits of organic fertilizers are well-documented, particularly in enhancing soil structure (Dogbatse et al.,2021) increasing microbial activity (Piaszczyk et al., 2017; Juknevičienė et al., 2019; van der Sloot et al.,2022) and providing a slow-release of nutrients (Cayuela et al., 2010), which can lead to more sustainable plant growth and development. The HHM, not only supply N but also improve the organic matter content and C/N ratio of the soil (Table 1), creating a more favorable environment for root development. These advantages may explain the superior biomass of lateral roots observed in cacaos fertilizers with HHM. In contrast, synthetic fertilizers, while providing a quick nutrient boost, may not contribute to the long-term health of the soil-plant-microbe system, potentially leading to less sustainable outcomes over time

The rationale behind this finding likely lies in the interaction within the soil-plant-microbe system. Bossolani et al. (2023) demonstrated that nitrogen input from synthetic fertilizers, such as ammonium sulfate, can alter the relationship between plant growth, soil factors, and microbial activity, particularly in systems previously amended with organic fertilizers. The C/N ratio of organic amendments plays a critical role in this shift. In this study, the application of urea likely influenced the dynamics between cacao root biomass and soil factors differently than HHM treatments considering: i) that the value of 1.26% of OM from the soil of the experimental site was not the optimal for cacao development (Hartemink, 2015; van Vliet & Giller, 2017), ii) The content of 94.9 % of total organic matter and iii) the 3.66 C/N ratio of HHM (Table 1).

3.3. Soil Plant Analysis Development (SPAD)

Relationship between SPAD values and chlorophyll concentration has been investigated in different species such as: *Arabidopis thaliana* L. (Ling et al.,2011), *Oryza sativa* L. (Yun et al., 2016), *Cannabis sativa* L. (Rodríguez-Yzquierdo et al., 2021), *Mangifera indica* L. (Ahmad et al., 2023), *Theobroma cacao* L. (Prastowo et al., 2021, Mensah et al., 2022). The variability in SPAD content is presumed result from structural differences among leaves of different species, which cause varying light reflection and scattering effects (Ling et al., 2011).

This variability is also influenced by shade and sun exposure in plantations (Mensha et al., 2022). In cacao crops, chlorophyll content is additionally influenced by genotype acclimatization capacity to environmental and soil conditions (Héctor-Ardisana et al., 2018). Furthermore, it has been demonstrated that the rate of photosynthesis (P_N) in cacao is associated with stomatal conductance and leaf nitrogen levels, and this varies among different genotypes (Daymond et al., 2011).

It is important to note that accurate recording of chlorophyll concentration values requires calibration equation tailored specifically to the species of interest (Ling et al., 2011). These considerations may explain the r value obtained (Figure 4) which is not high enough to be consider as a robust correlation, nevertheless, the value falls within the range of positive correlation, this weak correlation was also observed in the trial conducted by Prastowo et al. (2021).

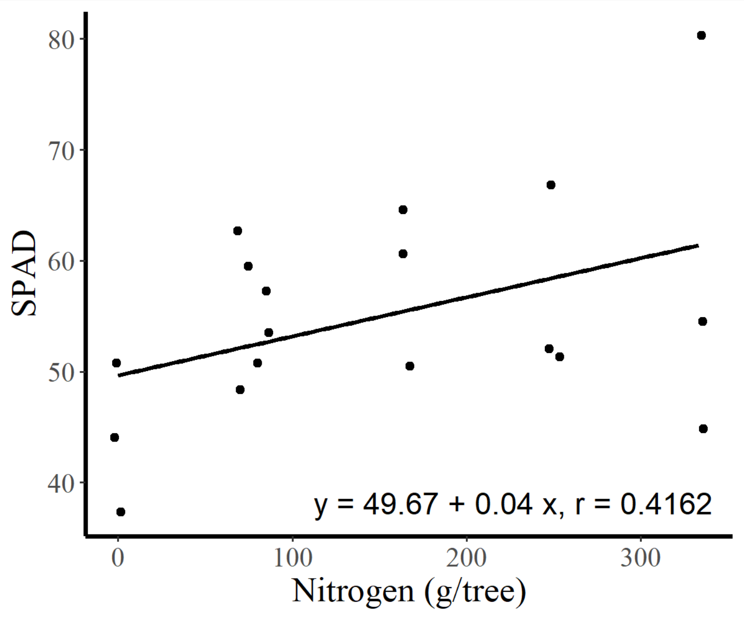

Figure 4. Linear regression of SPAD values against nitrogen doses per tree N (g tree^{-1}) from January 2020 (following completion of the four amendments). The intercept, 49.67, represents the expected SPAD value when the nitrogen dose is 0, while the slope of 0.04 indicates the rate of change in SPAD values for each unit increase in nitrogen dose (i.e., for every unit increase in nitrogen dose, the SPAD value increases by 0.04 units

It was observed a similar effect, no significant (P = 0.0503), between T2, T3, T4 and T5 for July 2019 (Figure 5). However, the highest value of SPAD was recorded in T5 (150 kg N ha^{-1} of urea), which was significantly different (P = 0.0013) from both T0 (control) and T1 (200 kg N ha^{-1}). Similarly, T2, T3 and T4 were also significantly different from (P = 0.0013) T0 and T1. As a result, the following subsets were stablished according to Scott-Knott test at *P < 0.05*: subset "a" (T5, T3, T4 and T2) and subset "b" (T1 and T0) (P = 0.013) with mean SPAD values of 62.68, 60.62, 57.26, 51.33, 44.83 and 37.3.

This may be explained by the lack of nitrogen in the amendment formulation for T0, as leaf chlorophyll concentration serves as an indicator of leaf nitrogen (Lin et al., 2011), and the time required for nitrogen doses in T1 (332.5 g $tree^{-1}$) to undergo mineralization and become available for assimilation (Cayuela et al., 2010; van der Sloot et al., 2022) taking into account that the texture class of the soil in this trial is sandy clay.

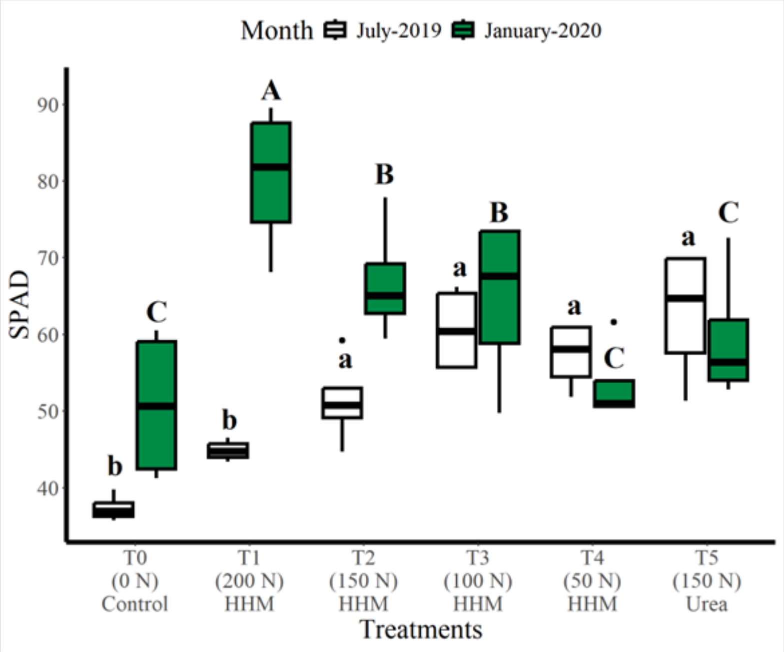

Figure 5. SPAD values in leaves of *Theobroma cacao* L. measured in July 2019 and March 2020. Data represent averages (*n* = 4) and error bars indicate standard error. Bars labeled with different letter within the same month are significantly different among treatments, as determined by Scott-Knott test at P < 0.05

Cayuela et al. (2010) noted in an incubation experiment, conducted without plants, that the content of ammonium (1900 mg kg^{-1}) from HHM transformed in nitrates (mg N kg^{-1} soil) were higher in a loam soil compared to a sandy soil in 35 days and 90 days after application. However, van der Sloot et al. (2022) noted that fertilizers with a C/N ratio of 10 or lower promote crop biomass growth in shorter times compared to those with high C/N ratios. Similarly, Bossolani et al. (2023) found that low C/N ratios in organic amendments enhance N mineralization, leading to improved crop growth. Consequently, the lower doses in T2 (249.9 g/tree), T3 (166.6 g/tree), and T4 (83.3 g/tree) performed better, given that the C/N ratio of HHM was 3.66 (Table 1).

In January 2020, an increase in media mean values was noted for T1 (79%), T0 (36%), T2 (30%), T3 (7%), while and T4 (-7%) and T5 (-5%) showed decreases. Significant differences were also observed (P = 0.00312) with treatments grouped differently compared to July 2019 nevertheless the treatments in each subset were different from for July 2019. Consequently, the following subsets were obtained according to the Scott-Knott test (P < 0.05): subset "A" (T1), subset "B" (T2 and T3) and subset "C" (T5, T4 and T0) (P = 0.023) (Figure 5), with mean SPAD values of 80.30, 66.81,64.59, 59.51, 53.53 and 50.75 respectively.

The reason why T1 were in the subset “A” may be attributed to the soil-plant-microbe system conditions where the necessary circumstances for nitrogen availability were achieved, given the slow-release properties of HHM (Delin & Engström, 2010; Cameron et al., 2013; van der Sloot et al., 2022). The increase in T0 could be explained by nutrient inputs from litterfall in the system (Fontes et al., 2014; Pérez-Flores et al., 2018; Afolayan, 2020; Asigbaase et al., 2021; Saj et al., 2021).

For instance, in cacao agroforest systems, leaf litter significantly contributes to soil nitrogen content, ranging from 84 to 174 kg of nitrogen per hectare annually (Pérez-Flores et al., 2018). Fontes et al. (2014) observed that cacao leaves alone accounted for 39% of nitrogen transfer from total leaf litterfall. However, they noted variations in nutrient return among agroforest systems, highlighting that nitrogen input from litterfall can vary depending on specific system characteristics (Fontes et al., 2014; Saj et al., 2021).

3.4. Leaf area (cm^2)

Under the specific soil conditions of the experimental site and the duration of the experiment, a similar effect between HHM and urea has been confirmed (Figure 6) (Aguirre, & Alegre, 2015). Thus, no significance difference was found for the month of July 2019 ($P = 0.0605$) and March 2020 ($P = 0.5181$). Despite this, the following rankings were established: T5>T4>T1>T0>T2>T3, with mean values of 219.76 cm^2, 202.18 cm^2, 186.42 cm^2, 184.68 cm^2, 178.13 cm^2, and 165.77 cm^2 for July 2019 and T2>T5>T1>T4>T3>T0 with mean values of 218.69 cm^2, 209.52 cm^2, 206.94 cm^2, 204.29 cm^2, 199.96 cm^2 and 197.12 cm^2 respectively for March 2020.

The percentage variation in the media of leaf area (cm^2) between July 2019 and March 2020 for T0, T1, T2, T3, T4 and T5 was: 7%, 11%, 23%,21%,1% and -5%. These results are similar than those from Prihastanti & Nurchayati (2022) who observed a non-significant difference for specific leaf area of cacao in three different plantation system considering that each have a different amount of input of nitrogen from the litterfall.

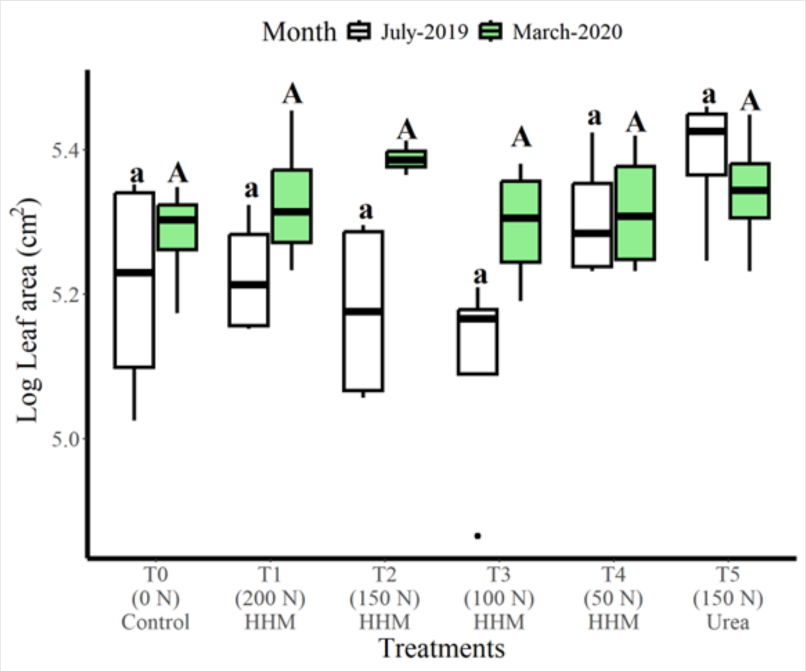

Figure 6. Leaf area (cm^2) log-transformation values in *Theobroma cacao* L. leaves measured in July 2019 and March 2020. Data represent averages ($n = 4$) and error bars indicate standard error. Bars labeled with different letter within the same month are significantly different among treatments, as determined by Scott-Knott test at $P < 0.05$

These findings may be attributed to: i) the influence of water on urea hydrolysis, enhancing its uptake by the plant and ii) the duration required for nitrogen in HHM (NH_4^+) to mineralize and be assimilated (Hartemink, 2005; Cayuela et al., 2010; van der Sloot et al., 2022).

In reference to point i) it is important to take into account the observations made by Cameron et al. (2013) regarding the process of urea hydrolysis, where factors such as atmospheric and soil temperature, soil moisture and pH levels, significantly influence this reaction. Throughout this chemical transformation, the urease enzyme acts as a catalyst; of urea where the resulting products such as ammonium and ammonia serve as an input for the growth and development of the plant (Taiz & Zeiger, 2006; Rodríguez-Yzquierdo et al., 2021; Zhu et al., 2021). Regarding point ii), sulfur present in the amino acids of HHM contributes to the formation of cross-linked protein bonds, reducing their solubility and degradability in water (Cayuela et al., 2010).

An additional factor to take into account is the time gap between the amendment month and the evaluation month. For example, in the evaluation for July 2019, there were six months and four months between the first and second amendments, respectively. In the case of March 2020, the four amendments were carried out with varying time intervals of 14 months, 12 months, eight months, and five months, respectively.

These time frames may explain the increase in the percent of mean values of T1, T2, T3 and T4 for March 2020, specially in T2 and T3 since HHM functions as an organic fertilizer with slow-release properties (Cayuela et al., 2010). Comparable findings were observed in the study conducted by Puentes et al. (2014), indicating that optimal agronomic efficiency in cacao cultivation can be achieved through the use of reduced dosages.

CONCLUSIONS

The application of horn and hoof meal (HHM), a non-conventional organic fertilizer derived from cattle by-products, demonstrated significant effects on *Theobroma cacao* L. development. Our findings indicate that the high nitrogen content—particularly in the form of NH_4^+—within HHM positively affected key biometric parameters, including SPAD content and the dry mass of lateral roots, in addition to boosting leaf area. Notably, lower doses of HHM (166.6 g tree^{-1} and 83.3 g tree^{-1}) demonstrated fertilization efficacy comparable to higher HHM rates and synthetic urea, especially in enhancing chlorophyll concentration: T2 (249.9 g tree^{-1}) and T3 (166.6 g tree^{-1}) surpassed the urea treatment (72.5 g tree^{-1}) by approximately 12% and 9%, and exceeded the control by about 32% and 27%, respectively. Furthermore, HHM-treated plants showed superior lateral root development compared to urea and control treatments; in particular, T4 (83.3 g tree^{-1}) achieved a 70% increase over urea and 56% over the control by the end of the experiment. These outcomes underscore the potential of HHM as an effective organic fertilizer for optimizing cacao plant physiology and productivity.

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CONFLICT OF INTEREST

There is no conflict of interest related to the material in the article.

AUTHORSHIP CONTRIBUTION

Conceptualization: Arévalo-Hernández, C. O.
Data curation: Arévalo-Hernández, C. O.
Formal analysis: Zumaeta-Barbarán, R. S.

Research: Arévalo-Hernández, C. O.
Methodology: Zumaeta-Barbarán, R. S. and Arévalo-Hernández, C. O.
Resources: Arévalo-Hernández, C. O.
Supervision: Zumaeta-Barbarán, R. S.
Validation: Arévalo-Hernández, C. O.
Visualization: Zumaeta-Barbarán, R. S.
Writing - original draft: Zumaeta-Barbarán, R. S. and Arévalo-Hernández, C. O.
Writing - review and editing: Zumaeta-Barbarán, R. S. and Arévalo-Hernández, C. O.

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