

Article Best Nitrogen Management Practices Using Sensor-Based Smart Agriculture in Nursery Production of Cacao

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Abstract: Reducing fertilizer costs and ensuring environmental sustainability are critical issues given the challenges posed by nutrient run-off. The use of smart technologies such as optical sensors is essential in achieving these goals. This study was conducted to determine the most efficient fertilizer regime based on chlorophyll content monitoring using optical sensor technology in cacao samplings in the nursery setting. The 8N-3P-9K (slow-released fertilizer) was used at 15 g (control), 15 g (supplemented with +15 g applied 2 times), 15 g (+15 g), 30 g (+15 g applied 2 times), 30 g (+15 g), and 45 g (+15 g applied two times). Chlorophyll content (measured using optical sensors such as soil plant analysis development (SPAD), atLEAF, normalized difference vegetation index (NDVI)), plant height, number of leaves, total nitrogen of leachate samples, and the total nitrogen and total carbon contents of the leaf and soil samples were evaluated over six months. The results show that the lower application of nitrogen fertilizer can provide the necessary required nutrients of cocoa plants and cause less contamination via run-off. Using 45 g (+15 g applied 2 times) causes more pollution through nutrient run-off. This study demonstrates the importance of handheld sensor technology in determining the best nitrogen management practices in fruit nurseries to reduce excessive fertilization while decreasing the extra costs and mitigating environmental pollution.

Keywords: *Theobroma cacao* L.; normalized difference vegetation index; SPAD-502 chlorophyll meter; atLEAF chlorophyll meter; chlorophyll content; run-off

1. Introduction

Cacao—also known as cocoa (*Theobroma cacao* L.)—is native to Central and South America. Currently, it is grown in 58 countries worldwide, covering over 17 million acres (6.9 million hectares) of land. This highly valuable crop significantly contributes to the global economy, with an annual worth exceeding USD 4 billion [1].

Cocoa plants are not commonly produced by nurseries in Florida; however, production for research purposes is being expanded in Southern Florida. Given the economic significance of cacao production, conducting research on nitrogen management practices is crucial for the successful cultivation of these valuable trees in Southern Florida.

Nitrogen (N) is a vital macronutrient in plant growth and development and is a core part of chlorophyll (Chl) in leaves. All growth parameters such as leaf weight and area, plant size, and transpiration rate can be affected by Chl levels. A low N content can cause deficiency symptoms that can affect plant quality, productivity, and most importantly, the salability. Excess N is also not desirable as it may cause N toxicity, stunted growth, and a low-quality plant. Most importantly, overfertilizing imposes additional costs on nursery producers and contributes to environmental hazards such as algal blooms due to nutrient run-off [2]. In general, one acre of land in nursery production has the potential to house up to 300,000 containers, many of which receive excessive fertilizer application. This is a primary driver in declining water quality throughout Florida, which ultimately contributes to algal blooms.



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). close to the plants/leaves [3,4]. Handheld sensors do not directly measure the N content in plant tissue but provide the radiation measurements or indirect measurements of indicator compounds that are sensitive to crop N status [5–7]. Optical devices determining the leaf N status through a non-destructive approach [6–8].

Optical sensors offer significant benefits on commercial farms, such as their ability to be employed at any stage of the growth cycle, low labor requirements, and compatibility with fertilizer decision-making procedures [9–11]. Certain sensors are restricted to single-point measurements, whereas others possess the capacity to continuously measure large representative surface areas of foliage. These attributes render them highly appropriate for pragmatic plant nitrogen status assessment [11–13].

Thus, the aim of this work was to determine the best nitrogen management practices by monitoring the chlorophyll content through optical sensors and smart agricultural technology. By employing this method, contamination through run-off can be minimized while simultaneously supplying sufficient nutrients for optimal plant growth.

2. Materials and Methods

Yellow cocoa seedlings which are approximately 60 cm in height were purchased from Santa Barbara Nursery (Miami, FL, USA) in September 2021. The one-year-old samplings were grown in a shade house at the Organic Garden at Florida International University (SW 17th St, Miami, FL 33174, USA). Initial 8N-3P-9K (slow-released fertilizer, Harrell's[®], Lakeland, FL, USA) was added in October, at control (15 g), T1 (15 g) + (15 g applied 2 times in November and March), T2 (15 g) + (15 g in November), T3 (30 g) + (15 g in November and March), T4 (30 g) + (15 g November), and T5 (45 g) + (15 g in November and March) (Table 1).

| Treatments | Fertilizer | Supplemented Fertilizer | Number and Month of Application |
|------------|------------|----------------------------|---------------------------------|
| Control | 15 g | 0 | |
| T1 | 15 g | 15 g | 2 times in November and March |
| T2 | 15 g | 15 g | 1 time in November |
| T3 | 30 g | 15 g | 2 times in November and March |
| T4 | 30 g | 15 g | 1 time on November |
| T5 | 45 g | 15 g | 2 times in November and March |

Table 1. Treatments, fertilizer amounts, and supplemented fertilizer amounts used.

The evaluations were conducted monthly for 6 months, represented by 0, 30, 60, 90, 120, 150, and 180 days after fertilization (DAF).

2.1. Number of Leaves, and Plant Height

Five plants were used per treatment and the number of leaves and plant height (cm) were measured in each pot.

2.2. Chlorophyll Content (Represented by SPAD Unit and atLEAF Unit), and Normalized Difference Vegetation Index (NDVI)

The readings were measured from five individual plants per treatment using optical sensors: GreenSeekerTM (normalized difference vegetation index—NDVI) (Trimble Agriculture, Sunnyvale, CA, USA), SPAD-502 chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan), and atLEAF chlorophyll meter (FT Green LLC, Wilmington, DE, USA). The sensor GreenSeekerTM that measures NDVI (Figure 1a) was set 45 cm above the plant canopy for the readings. For the SPAD and atLEAF (Figure 1b,c), the readings were collected from at least four leaves from the middle of the plant.



Figure 1. Optical sensors readings: (a) NDVI; (b) SPAD; and (c) atLEAF.

2.3. Leachate Samples

Individual plants were analyzed to evaluate leachate nutrient levels. Each plant was irrigated until soil saturation. After 30 min trays were placed underneath each plant pot, then the plants were further irrigated with 350 mL of water, allowing for the collection of 50 mL of leachate 5 min later. Samples were immediately refrigerated at 4 °C, then analyzed for total nitrogen (ppm) in CAChE Nutrient Analysis Core Facility at the Florida International University. The electrical conductivity (EC), pH, and salt concentration of each leachate sample was measured in-situ with a pH/conductivity meter (ExStik EC500, EXTECH Instrument, FLIR Commercial Systems, Goleta, CA, USA).

2.4. Nitrogen and Carbon Content of Substrate and Leaf Samples

The leaf samples of five plants per treatment were collected monthly by five plants per treatment. The substrate samples of five plants were collected at the beginning and at the end of this study. Samples were dried at 70 °C for 48 h, ground, and the total carbon (%), and total nitrogen (%) were analyzed in the CAChE Nutrient Analysis Core Facility at Florida International University.

2.5. Statistical Analysis

The experiment, consisting of six treatments, was replicated 5 times (one plant in each pot), with 30 plants in total in a completely randomized design (CRD). Data were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's test ($p \le 0.05$) using the SISVAR statistical program [14]. A correlation analysis was performed between means of chlorophyll content (atLEAF, SPAD, NDVI), total nitrogen, total carbon of leaf samples, and number of leaves with using the GraphPad Prism version 9.4.1, San Diego, CA, USA.

3. Results

3.1. Chlorophyll Content (Represented by SPAD Unit, and atLEAF Unit), Normalized Difference Vegetation Index (NDVI), Number of Leaves (NL), and Plant Height

The growth characteristics and the sensor parameters did not differ significantly ($p \le 0.05$) for the interaction between fertilization rate and days after fertilization (DAF). Therefore, these factors were evaluated separately.

Fertilizer treatments were not significantly different ($p \le 0.05$) in the number of leaves, SPAD, and NDVI. However, the atLEAF and plant height values were significantly different ($p \le 0.05$). The T5 (45 g) + (15 g in November and March) provided an increase in plant height (114) compared to T2 (15 g) + (15 g in November) (97.5), and T4 (30 g) + (15 g in November) (95.7). The T3 (30 g) + (15 g in November and March) provided an increase in

the chlorophyll content (atLEAF) (51.1) compared to T4 (30 g) + (15 g in November) (46.8) (Table 2).

| Treatments | SPAD | atLEAF | NDVI | NL | Plant Height (cm) |
|------------|-------------------|--------------------|--------------------|-------------------|----------------------|
| Control | 44.3 ^a | 50.1 ^{ab} | 0.800 ^a | 30.8 ^a | 109 ^{ab} |
| T1 | 44.4 ^a | 49.1 ^{ab} | 0.810 ^a | 30.8 ^a | 105 ^{ab} |
| T2 | 41.5 ^a | 47.9 ^{ab} | 0.800 ^a | 28.4 ^a | 97.5 ^b |
| T3 | 44.3 ^a | 51.1 ^a | 0.810 ^a | 27.1 ^a | 105 ^{ab} |
| T4 | 40.3 ^a | 46.8 ^b | 0.800 ^a | 33.0 ^a | 95.7 ^b |
| T5 | 43.8 ^a | 49.5 ^{ab} | 0.830 ^a | 30.0 ^a | 114 ^a |

Table 2. Chlorophyll content (represented by SPAD and atLEAF), normalized difference vegetation index (NDVI), number of leaves (NL), plant height of cocoa under different fertilization rate.

Means followed by the same letter within columns are not significantly different by Tukey's test ($p \le 0.05$). Control (15 g), T1 (15 g) + (15 g applied 2 times in November and March), T2 (15 g) + (15 g in November), T3 (30 g) + (15 g in November) and T5 (45 g) + (15 g in November and March).

An increase in the chlorophyll content (represented by atLEAF and SPAD) (49.7 to 53.0), and (44.7 to 46.4), respectively) was observed at 60, 90, 120, 150, and 180 DAF compared to 0 DAF (42.3 and 35.3), and 30 DAF (46.2, and 40.6). Thirty days after fertilization provided higher values (46.2 and 40.6) than day 0 (base reading) (42.3 and 35.3) to the same variables (Table 3).

Table 3. Chlorophyll content (represented by the SPAD unit and atLEAF unit), normalized difference vegetation index (NDVI), number of leaves (NL), and plant height of cocoa of 0–6 months.

| DAF | SPAD | atLEAF | NDVI | NL | Plant Height (cm) |
|-----|-------------------|-------------------|--------------------|-------------------|----------------------|
| 0 | 35.3 ^c | 42.3 ^c | 0.770 ^a | 17.3 ^d | 62.6 ^e |
| 30 | 40.6 ^b | 46.2 ^b | 0.820 ^a | 23.8 ^c | 76.0 ^d |
| 60 | 44.7 ^a | 49.7 ^a | 0.830 ^a | 25.9 ^c | 89.3 ^c |
| 90 | 45.3 ^a | 50.9 ^a | 0.820 ^a | 32.4 ^b | 109 ^b |
| 120 | 44.4 ^a | 50.6 ^a | 0.800 ^a | 31.3 ^b | 118 ^b |
| 150 | 44.9 ^a | 50.7 ^a | 0.820 ^a | 40.1 ^a | 136 ^a |
| 180 | 46.4 ^a | 53.0 ^a | 0.820 ^a | 39.5 ^a | 139 ^a |

Means followed by the same letter within columns are not significantly different by Tukey's test ($p \le 0.05$). 0–6 months represented by 0, 30, 60, 90, 120, 150, and 180 days after fertilization (DAF).

The highest number of leaves and plant height were recorded at 150 (40.1 and 136), and 180 DAF (39.5, and 139), respectively, indicating an increase in plant growth after fertilization. The increase in plant growth is accompanied by an increase in chlorophyll content (represented by SPAD and atLEAF) over time, showing the normal growth of the plants (Table 3).

3.2. Total Carbon (TC) and Total Nitrogen (TN) of Substrate and Leaf Samples

There was an interaction ($p \le 0.05$) between the fertilization rate and days after fertilization for total nitrogen and the total carbon of the substrate samples. An increase in total nitrogen (1.56) was observed in the T5 (45 g) + (15 g in November and March) at 180 days after fertilization and for total carbon, the increase (44.5) was observed in the treatment control (15 g) at 180 DAF (Table 4).

There was a significant interaction ($p \le 0.05$) between the fertilization rate and days after fertilization for total nitrogen, and the total carbon of the leaf samples. An increase in total nitrogen (2.51) was observed in the T3 (30 g) + (15 g in November and March) at 150 days after fertilization, and for total carbon the increase (48.2) was observed in T1 (15 g) + (15 g in November and March) at 150 DAF (Table 5).

| | Days after Fertilization (DAF) | | | | | | |
|------------|--------------------------------|-----------------------|--------------------|---------------------|-----------------------|--------------------|--------------------|
| Treatments | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | TN (%) | | | |
| Control | 1.62 ^{aC} | 1.60 ^{cD} | 1.59 ^{cE} | 1.55 ^{dF} | 1.74 ^{cA} | 1.72 ^{fB} | 1.00 ^{eG} |
| T1 | 1.62 ^{aB} | 1.38 fF | 1.48 ^{eD} | 1.49 ^{eC} | $1.45 ^{\mathrm{fE}}$ | 2.01 cA | 0.89 ^{fG} |
| T2 | 1.62 ^{aD} | 1.57 ^{eF} | 1.74 ^{bC} | 1.77 ^{bB} | 1.60 ^{dE} | 2.11 ^{bA} | 1.05 ^{dG} |
| Т3 | 1.62 ^{aF} | 1.74 ^{bD} | 1.56 ^{dG} | 1.97 ^{aC} | 2.20 ^{aB} | 2.51 ^{aA} | 1.67 ^{aE} |
| T4 | 1.62 ^{aD} | 1.59 ^{dE} | 1.59 ^{cE} | 1.64 ^{cC} | 1.79 ^{bB} | 1.95 ^{dA} | 1.15 ^{cF} |
| T5 | 1.62 ^{aD} | 1.93 ^{aA} | 1.93 ^{aA} | 1.77 ^{bC} | 1.55 ^{eE} | 1.81 ^{eB} | 1.47 ^{bF} |
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | TC (%) | | | |
| Control | 46.2 ^{aC} | 44.6 ^{cD} | 38.2 ^{eF} | 43.7 ^{fE} | 46.5 ^{aA} | 46.2 fB | 38.1 ^{bG} |
| T1 | 46.2 ^{aC} | 47.4 ^{aB} | 41.6 ^{cF} | 44.9 ^{eE} | 45.0 ^{dD} | 48.2 ^{aA} | 35.4 ^{cG} |
| T2 | 46.2 ^{aB} | 44.1 ^{dE} | 41.9 ^{bF} | 45.7 ° ^C | 44.7 ^{eD} | 47.9 ^{bA} | 35.3 ^{dG} |
| Т3 | 46.2 ^{aB} | 45.9 ^{bD} | 34.6 fF | 46.0 ^{bC} | 45.4 ^{cE} | 46.8 ^{dA} | 34.3 ^{eF} |
| T4 | 46.2 ^{aD} | $41.5 ^{\mathrm{fE}}$ | 41.5 ^{dF} | 47.2 ^{aB} | 46.3 ^{bC} | 47.5 ^{cA} | 38.2 ^{aG} |
| T5 | 46.2 ^{aB} | 42.1 ^{eE} | 42.1 ^{aE} | 45.5 ^{dC} | 44.3 ^{fD} | 46.7 ^{eA} | 31.5 ^{fF} |

Table 4. Total nitrogen (TN) and total carbon (TC) of substrate samples of cocoa plants under different fertilization rate of 0–6 months.

Means followed by the same lower case letters in the columns (treatments) and upper case letters in the rows (DAF) are not significantly different according to Tukey's test ($p \le 0.05$). The control (15 g), T1 (15 g) + (15 g applied 2 times in November and March), T2 (15 g) + (15 g in November), T3 (30 g) + (15 g in November and March), T4 (30 g) + (15 g in November) and T5 (45 g) + (15 g in November and March). 0 and 6 months represented by 0 and 180 days after fertilization (DAF).

Table 5. Total carbon (TC) and total nitrogen (TN) and of leaf samples of cocoa plants under different fertilization rate of 0–6 months.

| | | | | DAF | | | |
|------------|--------------------|-----------------------|--------------------|--------------------|-------------------------|--------------------|--------------------|
| Treatments | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | TC (%) | | | |
| Control | 46.2 ^{aC} | 44.6 ^{cD} | 38.2 ^{eF} | 43.7 ^{fE} | 46.5 ^{aA} | 46.2 ^{fB} | 38.1 ^{bG} |
| T1 | 46.2 ^{aC} | 47.4 ^{aB} | 41.6 ^{cF} | 44.9 ^{eE} | 45.0 ^{dD} | 48.2 ^{aA} | 35.4 ^{cG} |
| T2 | 46.2 ^{aB} | 44.1 ^{dE} | 41.9 ^{bF} | 45.7 ^{cC} | 44.7 ^{eD} | 47.9 ^{bA} | 35.3 ^{dG} |
| T3 | 46.2 ^{aB} | 45.9 ^{bD} | 34.6 ^{fF} | 46.0 ^{bC} | 45.4 ^{cE} | 46.8 ^{dA} | 34.3 ^{eF} |
| T4 | 46.2 ^{aD} | $41.5 ^{\mathrm{fE}}$ | 41.5 ^{dF} | 47.2 ^{aB} | 46.3 ^{bC} | 47.5 ^{cA} | 38.2 ^{aG} |
| T5 | 46.2 ^{aB} | 42.1 ^{eE} | 42.1 ^{aE} | 45.5 ^{dC} | 44.3 ^{fD} | 46.7 ^{eA} | 31.5 fF |
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | TN (%) | | | |
| Control | 1.62 ^{aC} | 1.60 ^{cD} | 1.59 ^{cE} | 1.55 ^{dF} | 1.74 ^{cA} | 1.72 ^{fB} | 1.00 ^{eG} |
| T1 | 1.62 ^{aB} | 1.38 ^{fF} | 1.48 ^{eD} | 1.49 ^{eC} | $1.45 \ ^{\mathrm{fE}}$ | 2.01 ^{cA} | 0.89 ^{fG} |
| T2 | 1.62 ^{aD} | 1.57 ^{eF} | 1.74 ^{bC} | 1.77 ^{bB} | 1.60 ^{dE} | 2.11 ^{bA} | 1.05 ^{dG} |
| Т3 | 1.62 ^{aF} | 1.74 ^{bD} | 1.56 ^{dG} | 1.97 ^{aC} | 2.20 ^{aB} | 2.51 ^{aA} | 1.67 ^{aE} |
| T4 | 1.62 ^{aD} | 1.59 ^{dE} | 1.59 ^{cE} | 1.64 ^{cC} | 1.79 ^{bB} | 1.95 ^{dA} | 1.15 ^{cF} |
| T5 | 1.62 ^{aD} | 1.93 ^{aA} | 1.93 ^{aA} | 1.77 ^{bC} | 1.55 ^{eE} | 1.81 ^{eB} | 1.47 ^{bF} |

Means followed by the same letter lower case in the columns (Treatments) and upper case in the rows (DAF) are not significantly different by Tukey's test ($p \le 0.05$). Control (15 g), T1 (15 g) + (15 g applied 2 times in November and March), T2 (15 g) + (15 g in November), T3 (30 g) + (15 g in November and March), T4 (30 g) + (15 g in November) and T5 (45 g) + (15 g in November and March). 0–6 months represented by 0, 30, 60, 90, 120, 150, and 180 days after fertilization (DAF).

3.3. Electric Conductivity (EC), Salt, and Total Nitrogen (TN) of Leachate Samples

There was an interaction ($p \le 0.05$) between the fertilization rate and days after fertilization for salt, electric conductivity (EC), and total nitrogen (TN) of leachate samples. An increase in salt and EC (3938 and 7156), respectively, was observed in the T5 (45 g) + (15 g in November and March) at 30 days after fertilization, and for TN, increases (377 and 337) were observed at 30 DAF and 60 DAF, respectively, using the same treatment (Table 6).

Table 6. Electric conductivity (EC), salt, and total nitrogen (TN) of the leachate samples of cocoa plants under different fertilization rates from 0 to 6 months.

| | | | | DAF | | | |
|------------|--------------------|---------------------|----------------------|---------------------|--------------------------|--------------------|-----------------------|
| Treatments | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | EC (µs) | | | |
| Control | 631 ^a | 3134 ^d | 2071 ^c | 1541 ^c | 663 ^a | 780 ^b | 750 ^c |
| T1 | 631 ^{aE} | 4032 cdA | 3820 ^{bAB} | 2793 ^{bBC} | 941 ^{aDE} | 2258 ^{aC} | 1858 ^{abCD} |
| T2 | 631 ^{aB} | 4632 bcA | 4978 ^{aA} | 1105 ^{cB} | 1021 ^{aB} | 907 ^{bB} | 671 ^{cB} |
| T3 | 631 ^{aD} | 5710 ^{bA} | 5376 ^{aAB} | 4350 ^{aB} | 1018 ^{aD} | 2456 ^{aC} | 2323 ^{aC} |
| T4 | 638 ^{aC} | 5574 ^{bA} | 5444 ^{aA} | 4004 ^{aB} | 1170 ^{aC} | 1003 ^{bC} | 849 bcC |
| T5 | 638 ^{aF} | 7156 ^{aA} | 5544 ^{aB} | 4404 ^{aC} | $1534 \ ^{\mathrm{aEF}}$ | 2930 ^{aD} | 1941 ^{abDE} |
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | Salt (ppm) | | | |
| Control | 303 ^{aC} | 1616 ^{dA} | 1057 ^{dAB} | 761 ^{cBC} | 309 ^{aC} | 377 ^{bC} | 362 ^{bC} |
| T1 | 303 ^{aD} | 2170 ^{cdA} | 2020 cAB | 1454 ^{bBC} | 453 ^{aD} | 1159 ^{aC} | 940 ^{abCD} |
| T2 | 303 ^{aB} | 2514 ^{bcA} | 2704 ^{bA} | 544 ^{cB} | 498 ^{aB} | 441 ^{bB} | 358 ^{bB} |
| T3 | 303 aD | 3080 ^{bA} | 2934 ^{abAB} | 2338 ^{aB} | 506 ^{aD} | 1302 ^{aC} | 1191 ^{aC} |
| T4 | 303 aC | 3044 ^{bA} | 2968 ^{abA} | 2138 aB | 512 ^{aC} | 489 ^{bC} | 415 ^{bC} |
| T5 | 321 ^{aD} | 3938 ^{aA} | 3412 ^{aA} | 2376 ^{aB} | 763 ^{aD} | 1638 ^{aC} | 973 ^{abD} |
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| - | | | | TN (ppm) | | | |
| Control | 12.4 ^{aB} | 125 ^{fA} | 125 ^{fA} | 3.17 ^{fF} | 11.3 ^f | 5.57 ^{eE} | 5.71 ^{eD} |
| T1 | 12.4 ^{aD} | 175 ^{eA} | 175 ^{eA} | 12.3 ^{eE} | 21.7 ^{eC} | 11.4 ^{cF} | 83.0 ^{bB} |
| T2 | 12.4 ^{aE} | 196 ^{dA} | 196 ^{dA} | 16.3 ^{dD} | 26.1 ^{bB} | 17.3 ^{bC} | 8.11 ^{dF} |
| Т3 | 12.4 ^{aE} | 292 ^{bA} | 292 ^{bA} | 19.0 ^{cD} | 23.8 ^{cC} | 9.50 ^{dF} | 85.2 ^{aB} |
| T4 | 12.4 ^{aE} | 275 ^{cA} | 275 ^{cA} | 19.7 ^{bD} | 22.8 ^{dC} | 83.0 ^{aB} | $5.40 ^{\mathrm{fF}}$ |
| T5 | 12.4 ^{aE} | 377 ^{aA} | 337 ^{aA} | 19.8 ^{aD} | 40.3 ^{aB} | 2.41 ^{fF} | 37.8 ^{cC} |

Means followed by the same letter lower case in the columns (Treatments) and upper case in the rows (DAF) are not significantly different according to Tukey's test ($p \le 0.05$). Control (15 g), T1 (15 g) + (15 g applied 2 times in November and March), T2 (15 g) + (15 g in November), T3 (30 g) + (15 g in November and March), T4 (30 g) + (15 g in November) and T5 (45 g) + (15 g in November and March). 0–6 months represented by 0, 30, 60, 90, 120, 150, and 180 days after fertilization (DAF).

3.4. Correlation between Chlorophyll Content (Indicated by SPAD and atLEAF), Normalized Difference Vegetation Index (NDVI), Number of Leaves (NL), and Total Nitrogen (TN) and Total Carbon (TC) of Leaf Samples

The association between SPAD and atLEAF was evidenced by a correlation analysis, in which significant and high correlation ($p \le 0.05$) were observed at 30 DAF (0.861), 150 DAF (0.870), and 180 DAF (0.869). Significant and high correlations ($p \le 0.01$) were also observed between the total nitrogen and total carbon at 60 DAF (0.915), and between NDVI and the number of leaves at 90 DAF (0.926) (Table 7).

| | atLEAF | NDVI | TN (%) | TC (%) | NL | | | |
|--------|---------|--------|--------|-----------|----------|--|--|--|
| 30 DAF | | | | | | | | |
| SPAD | 0.861 * | 0.777 | -0.205 | 0.382 | 0.121 | | | |
| atLEAF | | 0.418 | -0.023 | 0.343 | 0.419 | | | |
| NDVI | | | -0.536 | 0.441 | -0.446 | | | |
| TN (%) | | | | -0.909 ** | 0.217 | | | |
| TC (%) | | | | | 0.160 | | | |
| | | 60 I | DAF | | | | | |
| SPAD | 0.683 | 0.440 | -0.254 | -0.321 | 0.018 | | | |
| atLEAF | | 0.528 | -0.201 | -0.132 | -0.152 | | | |
| NDVI | | | 0.387 | 0.118 | 0.186 | | | |
| TN (%) | | | | 0.915 ** | 0.272 | | | |
| TC (%) | | | | | 0.147 | | | |
| | | 90 I | DAF | | | | | |
| SPAD | 0.496 | -0.146 | -0.635 | -0.359 | -0.477 | | | |
| atLEAF | | 0.233 | -0.229 | -0.532 | -0.018 | | | |
| NDVI | | | -0.294 | -0.384 | 0.926 ** | | | |
| TN (%) | | | | 0.795 | -0.064 | | | |
| TC (%) | | | | | -0.183 | | | |
| | | 1201 | DAF | | | | | |
| SPAD | 0.591 | -0.307 | 0.655 | 0.636 | -0.364 | | | |
| atLEAF | | -0.446 | -0.048 | 0.142 | -0.886 * | | | |
| NDVI | | | -0.032 | -0.064 | 0.321 | | | |
| TN (%) | | | | 0.711 | 0.407 | | | |
| TC (%) | | | | | 0.226 | | | |
| | | 150 | DAF | | | | | |
| SPAD | 0.870 * | -0.190 | -0.206 | 0.132 | -0.750 | | | |
| atLEAF | | -0.258 | -0.345 | -0.106 | -0.854 * | | | |
| NDVI | | | 0.474 | -0.667 | 0.316 | | | |
| TN (%) | | | | -0.468 | -0.030 | | | |
| TC (%) | | | | | 0.243 | | | |
| | | 180 | DAF | | | | | |
| SPAD | 0.869 * | 0.342 | 0.174 | 0.630 | -0.349 | | | |
| atLEAF | | 0.218 | 0.092 | 0.381 | -0.095 | | | |
| NDVI | | | 0.725 | -0.042 | 0.554 | | | |
| TN (%) | | | | 0.291 | 0.620 | | | |
| TC (%) | | | | | -0.492 | | | |

Table 7. Correlation coefficient (r) for chlorophyll content (indicated by SPAD unit and atLEAF unit), normalized difference vegetation index (NDVI), number of leaves (NL), and total nitrogen (TN) and total carbon (TC) of leaf samples in cocoa of 0–6 months.

Pearson's correlation coefficient (r) significant at $p \le 0.05$ (*), $p \le 0.01$ (**). 0–6 months represented by 0, 30, 60, 90, 120, 150, and 180 days after fertilization (DAF).

Correlation analysis showed a significant ($p \le 0.05$) negative correlation between total nitrogen and total carbon at 30 DAF (-0.909). Additionally, significant negative ($p \le 0.01$) correlations were observed between atLEAF and the NL at 120 DAF (-0.886), and 150 DAF (-0.854) (Table 7).

4. Discussion

As a vital component of metabolism in plants and the core of amino acids, proteins, enzymes, nucleic acids, chlorophylls, and hormones, nitrogen is an essential nutrient for all plants. Consequently, any deficiency in nitrogen rapidly impedes plant growth [15]. Following the application of N fertilizers to the soil, there are several possible pathways through the cacao agricultural ecosystem, such as rapid uptake by the trees, or leaching and/or volatilization [16]. Producers are using a large number of pesticides, fertilizers and herbicides to achieve higher production, often using larger doses than standard or optimum for these products. Higher doses of fertilizers accelerate the pollution of the air and water [17].

It is therefore essential to align the nitrogen use with crop demand to be able to optimize N fertilizer applications [12,18]. A highly effective strategy could involve promptly and frequently assessing the nitrogen status of crops on the farm, allowing for swift adjustments to the nitrogen supply [3,19,20]. Proximal optical sensors represent a diverse range of non-invasive monitoring instruments that can be utilized to evaluate the nitrogen status of crops [3,5,21]. The parameters that were measured in this experiment were aimed at establishing optimal fertilizer application rates for cocoa as a reference for nursery producers. Additionally, the data collected can be utilized to determine the suitability of SPAD, atLEAF, and GreenSeekerTM devices in estimating fertilizer application rates based on leaf nitrogen measurements. Wang et al. [22] found that both handheld sensors (SPAD meter and pocket NDVI unit) were capable of indicating the status of canopy variables related to N for two geranium cultivars. Hardin [23] observed that the SPAD meter provided a good indication of leaf N concentration in selected cultivars of pecan. Dunn and Goad [24] noted that either sensor (SPAD or atLEAF) can be used for correlating the leaf N in ornamental cabbage plants.

According to what was observed in this study, there was no significant difference between treatments ($p \le 0.05$) for the number of leaves, normalized difference vegetation index (NDVI), and the relative chlorophyll content by SPAD. This can be due to insufficient differences in biomass between plants which observed in ornamental cabbage and *Justicia brandegeana* [24,25]. These results differ from those of Khoddamzadeh and Dunn [25], who found significant differences in NDVI and SPAD values among the rates of fertilizer application when testing at different dates after fertilizer treatment during the vegetative stage in two Chrysanthemum cultivars.

However, significant differences between treatments were found for plant height and chlorophyll content by atLEAF in the current study. Using 45 g (+15 g November and March; 5) provided an increase in plant height (114) compared to 15 g (+15 g November; T2) (97.5), and 30 g (+15 g November; T4) (95.7). The 30 g (+15 g November and March; T3) treatment provided an increase in the relative chlorophyll content (atLEAF) (51.1) compared to 30 g (+15 g November; T4) (46.8) (Table 2). An important issue with using cocoa leaf analysis is that the nutrient content of cocoa can be affected by many factors, such as age and leaf development stage, fruit bearing, light intensity, and most importantly seasonal effects [26].

An increase in total nitrogen (2.51) in the leaf samples was observed in the 30 g (+15 g November and March; T3) treatment at 150 days after fertilization, and for total carbon the increase (48.2) was observed using 15 g (+15 g November and March; T1) at 150 DAF (Table 4). Plant tissue analysis has been used as a reference technique to estimate the plant N status [27,28]. These results show that the 30 g (+15 g November and March; T3) treatment provided greater N absorption, as well as an increase in the relative chlorophyll by atLEAF content compared to the 30 g (+15 g November; T4) treatment.

An increase in total nitrogen (1.56) in the soil samples was observed in the 45 g (+15 g November and March; 5) treatment at 180 days after fertilization, and for the total carbon, the increase (44.5) was observed using 15 g (control) at 180 DAF (Table 5). These results show that, in the treatment 45 g (+15 g November and March; 5), not all N was absorbed by the plant, and the nitrogen that remains in the soil can easily be lost in run-off. In fact, this is what was observed in the leachate sample results. An increase in salt and EC (3938 and 7156), respectively, was observed in the treatment 45 g (+15 g November and March; 5) at 30 days after fertilization, and for TN, increases (377 and 337) were observed at 30 and 60 DAF, respectively, using the same treatment (Table 6). Therefore, the highest dose of fertilizer, the 45 g (+15 g November and March; 5) treatment, provided the highest run-off compared to the other treatments, and with regard to growth parameters, chlorophyll content, and NDVI, the results were similar.

The association between the SPAD and atLEAF sensors at 30, 150, and 180 DAF was evident in the correlation analysis; this means that either sensor can be used to measure the chlorophyll content. Working with the fertility status in ornamental cabbage, Dunn and Goad [24] indicated that the atLEAF and SPAD readings were different but correlated. The same authors noted an average reading difference of 5.5 between the two sensors, with atLEAF always producing higher readings. A similar result was observed in this experiment.

It is important to highlight the association between the normalized difference vegetation index (NDVI) and number of leaves (NL) at 90 DAF. This correlation demonstrates that NDVI is positively related to the NL, i.e., when NL increases, so does the NDVI, or vice versa. Khoddamzadeh and Dunn [25] worked with nitrogen management in Chrysanthemum and observed that the NDVI values were more strongly correlated with leaf N than SPAD and atLEAF chlorophyll sensors. These results were different from this study, where none of the sensors showed a correlation with atLEAF represented by the total nitrogen (%) in leaf samples.

Negative correlations were observed between the total nitrogen and total carbon at 30 DAF, and between atLEAF and the number of leaves at 120 and 150 DAF, indicating that, with a linear increase in one parameter, the other parameter decreases.

This research provides a foundation for future studies and nursery practices by highlighting the significance of nitrogen-monitoring technologies such as optical sensors. This study reveals that lower nitrogen fertilizer treatments can provide adequate nutrients for the growth of cocoa plants (Figure 2), while causing less nutrient run-off contamination. Similar results were observed by Costa et al. [29] in their study of nitrogen fertilization of cocoplum plants based on the monitoring of chlorophyll content through optical sensors.



Figure 2. Cocoa plants under different fertilization rates at six months of the experiment. Control (15 g), T1 (15 g) + (15 g applied 2 times in November and March), T2 (15 g) + (15 g in November), T3 (30 g) + (15 g in November and March), T4 (30 g) + (15 g November) and T5 (45 g) + (15 g in November and March).

It is important to highlight that more studies must be carried out in order to clarify more about nitrogen management in cocoa plants in nursery settings, mainly studies aimed at the complete cycle of this crop.

5. Conclusions

All the lower treatments of nitrogen fertilizer provided nutrients in amounts necessary for the growth of cocoa plants, concurrently providing less pollution via nutrient run-off. Using 45 g (+15 g November and March; T5) provides higher contamination through nutrient run-off. This study serves as a basis for future studies and demonstrates the importance using smart agriculture and other technologies such as optical sensors in nitrogen monitoring in determining the best nitrogen management practices for fruit trees at nursery settings. This practice would equip them with the instant tools necessary to avoid excessive fertilization, thus avoiding extra costs and environmental damage in fruit nurseries. Author Contributions: Conceptualization, A.A.K. and B.N.S.C.; Methodology, A.A.K.; Software, B.N.S.C.; Validation, A.A.K. and B.N.S.C.; Formal Analysis, B.N.S.C.; Investigation, B.N.S.C.; Resources, A.A.K.; Data Curation, B.N.S.C.; Writing—Original Draft Preparation, A.A.K. and B.N.S.C.; Writing—Review and Editing, A.A.K. and B.N.S.C.; Visualization, B.N.S.C.; Supervision, A.A.K.; Project Administration, A.A.K.; Funding Acquisition, A.A.K. All authors have read and agreed to the published version of the manuscript.

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