Research Article

Influence of Tree Shade on the Growth and Chlorophyll Content of Arabica Coffee Plants Established in an Agroforestry System at Southern Manabí, Ecuador

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Abstract | The experiment was developed under production conditions on a farm in the Santa Ana city, south-central region of the province of Manabí, Ecuador, in a mountainous area of approximately 300 meters above sea level. Coffee (*Coffea arabica*, sp.) production is carried out in a context of peasant family agriculture, with an agroforestry system with coffee trees of the Sarchimor variety planted at 1.5 x 1.5 m, interspersed with tree species typical of the dry forest. The system involves manual weed control, without fertilization, irrigation, phytosanitary control, or shade regulation. In this scenario, and during an experimental period of 90 days (03/08/2022 - 26/10/2022), phenological variables of coffee trees maintained in a study area of 50 x 50 m at a high (S1: 51-70%) and low (S3: 1-30%) shade level was compared with those obtained at an intermediate shade level considered as standard (S2: 31-50%). The phenological variables related to vegetative development (Total Branches) of coffee plants showed higher values in S2 compared to S1 and S3. These results are related to the higher photosynthetic activity associated with the higher intensity of incident solar radiation, although the relationship is not linear. In our results, flowering and fruiting were not affected by the level of shade, nor were their precursors, such as nodes per productive branch and productive nodes per productive branch. On the other hand, coffee plants at full sun exposure in S1, without shade, decreased chlorophyll measured in SPAD units, as a possible compensation for the increase in photosynthetically active uptake in that condition.

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Introduction

The effect of tree shade on coffee (*Coffea* spp.) agroforestry systems (AFS) has been studied from different angles to establish its influence on the quantitative (Jaramillo-Botero *et al.*, 2010; Koutouleas *et al.*, 2022) and qualitative production of coffee beans (Duicela *et al.*, 2017; Geeraert *et al.*, 2019; Geromel *et al*., 2008; Koutouleas *et al*., 2022; Worku *et al*., 2022). The interest in this topic arises from the need to take advantage of diversified systems to mitigate the environmental impact of climate change (Burgess *et al*., 2022; Jha *et al*., 2014; Koutouleas *et al*., 2022; Raj *et al*., 2022) and as an alternative to the volatility of coffee prices in the market (Geeraert *et al*., 2019).

The AFS is recognized for its positive effects on the moderation of the thermohygrometric regime (Campanha *et al*., 2004; Geeraert *et al*., 2019; Lin, 2007), protection against erosion (Cannavo *et al*., 2011), fertility (Jaramillo-Botero *et al*., 2010), organic matter content, water holding capacity of soil (Padovan *et al*., 2018; Tumwebaze and Byakagaba, 2016), and its ability to reduce water stress in the face of climate change and its consequences on the rainfall regime (Cannavo *et al*., 2011). However, the decrease in photosynthetically active radiation that accompanies shade is physiologically linked to a concomitant reduction in the production precursors of Arabica coffee (*Coffea arabica* L.), when crops in full sun are compared with those kept under shade (Campanha *et al*., 2004; Jaramillo-Botero *et al*., 2010; Rapidel *et al*., 2015).

The reduction in photosynthetically active radiation is logically preceded by a decrease in vegetative growth rates (DaMatta, 2004), which causes a delay in the formation of plagiotropic branches (those that give rise to other secondary and tertiary branches) in coffee bushes (Jaramillo-Botero *et al*., 2010; Sarmiento *et al*., 2022), productive nodes, leaf area (Jaramillo-Botero *et al*., 2010), flowers, and fruits (Campanha *et al*., 2004). However, the decrease in the rate of vegetative growth improves the size and quality of the fruits (Jaramillo-Botero *et al*., 2010). It also helps moderate the effects of high radiation intensity in agroforestry systems, reducing the biennial fluctuation in quantitative coffee production. This phenomenon, known as seasonal biennially, is caused by the depletion of photoassimilate reserves, primarily in the root and stem, due to increased demand for flowers and fruits. This increased demand is, in turn, influenced by higher radiation intensity (Campanha *et al*., 2004; DaMatta, 2004). Consequently, a decrease in radiation in agroforestry systems is associated with a lower interannual fluctuation in coffee production. This reduction in fluctuation has a positive impact on the distribution and sales processes, as well as greater stability in the supply chain.

The organoleptic attributes of coffee, which ultimately define the qualities of the coffee beverage, represent a strategically important component of quality management and competitive differentiation in the market (Cordova and Valdivia, 2021). Significant improvements in organoleptic attributes that determine the quality of coffee has been reported when it comes from shaded AFS at different levels (Campanha *et al*., 2004; Geeraert *et al*., 2019; Jaramillo-Botero *et al*., 2010), as well as interactions with fertility (Bosselmann *et al*., 2009), temperature and humidity regime (Jaramillo-Botero *et al*., 2010), variety (Gonzales *et al*., 2023), and agroecosystem altitude (Guyot *et al*., 1996; Salazar *et al*., 2000).

Especially relevant are the favorable results obtained in coffee AFS at suboptimal altitudes (300-600 masl), comparable to those of coffee at altitude (1200-1600 masl), which suggests the possibility of using AFS as a formula to improve the competitive positioning of coffee in areas less favored by their relief conditions (Padovan *et al*., 2018). In Manabí, a coastal province of Ecuador, there is a long tradition in the production of coffee in AFS located in areas of rugged relief at low altitudes. Nevertheless, the absence of quality incentives threatens the sustainability of coffee in the face of more profitable alternatives from other crops whose vegetative cycles are less than one year and are replanted after harvest (short-cycle crops). Additionally, in Ecuador, the influence of tree shade on AFS has been reported early, but at classically recommended altitude (Duicela *et al*., 2017). However, there is no information on its effects at lower altitudes, which are those that prevail in the province of Manabí and, specifically, in its southern zone. With this background, it is proposed to investigate the effects of different levels of tree shade on the vegetative development of Arabica coffee bushes grown in an AFS at suboptimal heights and in the field conditions typical of peasant family farming in the southern zone of the province of Manabí, Ecuador.

Materials and Methods

The experiment was conducted under production conditions on a farm in the Santa Ana canton, in the central-southern region of the province of Manabí. The farm is situated in a hilly area with an altitude of approximately 300 masl. Coffee production (*C. arabica* L. var. Sarchimor) takes place in the context of peasant family farming, utilizing an agroforestry system. The system consists of coffee plants planted at 1.5 x 1.5 m spacing. These trees are interspersed with typical tree species found in dry forests (Table 1). Weed plants are manually controlled, and there is no fertilization, irrigation, phytosanitary control, or shade regulation.

Table 1: *Plant species present in the study area.*

Shade level	Tree species
S_1	*Pseudosamanea guachapele* (Kunth) Hams, *Erytrina poeppigiana* (Walp.) O.F. Cook, *Inga edulis* Mart., *Mangifera indica L, Citrus sinensis (L.)* Osbeek
S_2	*I. edulis*, *C. sinensis*, *Bombacopsis* sp., *Cordia macrantha* Chodat
S_3	*Erytrina* poeppigiana (Walp.) O.F. Cook, *I. edulis*, *Cedrela adorate* L.

For the report, a study area of 2500 m^2 (50 x 50 m) was selected. In this area, 70 trees were present, and the percentage of shade projected by each tree was estimated. The shade estimation was performed using a computer application called Shade Motion 4.0 (Somarriba *et al*., 2020). The application utilized in situ measurements of each tree, including treetop type, diameter at chest height, trunk height, treetop height, treetop width, and treetop density. These measurements were georeferenced with the coordinates of the trees in the study area, allowing for the representation of a map illustrating areas with homogeneous shade.

Initially, seven shade levels were identified. To investigate the effect of shade on phenological indicators correlated with quantitative coffee production, five coffee trees were randomly selected in each shade level. The shade levels ranged from 0 to 70%, with the following categories: 1: 0-10%, 2: 11-20%, 3: 21-30%, 4: 31-40%, 5: 41-50%, 6: 51-60%, and 7: 61-70%. The numbers 1-7 identify the zones of the study area with homogeneous shade levels, estimated and georeferenced using ShadeMotion 4.0. The shade levels in each zone were expressed as a percentage (%) compared to the shadeless zones under full sun exposure. This estimation was performed by ShadeMotion, using on-site measurements from each of the 70 trees in the study area as input. The intervals in each of the seven shade levels indicate the range of shade percentage variation.

It is generally recognized that shade effects are positive only at intermediate levels (Duicela *et al*., 2017; Jaramillo-Botero *et al*., 2010; Koutouleas *et al*., 2022; Netsere and Kufa, 2015). Therefore, a regrouping of shade levels was performed to facilitate comparison with a pattern close to the optimal range, specifically with a percentage of 31-50%. Consequently, the regrouped shade levels were as follows: S1: 0-30%, S2: 31-50%, and S3: 50-70% (Shade level -S-: Independent variable). Accordingly, there were not always the same amount of coffee trees at each shade level: S1 had 15 coffee trees, S2 had 10 coffee trees, and S3 had 10 coffee trees: S1 had 15 coffee trees, S2 had 10 coffee trees, and S3 had 10 coffee trees. These coffee trees were situated in different areas of the study area and were associated with trees of different species (**Table 1**).

The phenological variables measured in the middle (M) and lower (L) thirds of the coffee trees: total branches (TB-M and TB-L), total productive branches (TPB-M and TPB-L), nodes of a productive branch (NPB-M and NPB-L), productive nodes of a productive branch (PNPB-M and PNPB-L), and fruits or flowers in productive branches (FPB-M and FPB-L). In addition to these variables, the relative amount of chlorophyll, measured in SPAD units (C) with the SPAD-502 Plus (MCL502 Minolta SPAD 502 plus model e standard), was estimated by averaging the readings taken from four randomly selected leaves on each plant. Furthermore, the plant height (H) in meters (m) and the stem diameter at a point 15 centimeters above the ground (D) were measured, considering the findings of Segura *et al*. (2006).

These variables were recorded fortnightly for 90 days, from 03/08/2022 to 26/10/2022, resulting in a total of six readings. This timeframe introduced another source of independent variation (Time -T-: Independent Variable). The statistical analysis also included the examination of the T x S interaction on the dependent variables.

Statistical analysis

A descriptive analysis with boxplots was performed, showing the median, interquartile range, minimum, and maximum of the dependent variables by levels of shadow exposure (S). Furthermore, a Generalized Estimating Equation (GEE) with a Gaussian distribution was used to estimate the effect of S1 (0-30%), S2 (31-50%) and S3 (51-70%), on the various dependent variables (phenological variables) of the

35 coffee trees. These plants were measured six times as part of the experiment, with each measurement considered a repeated measure. Additionally, we considered the interaction between the level of shadow (S) and time (T). We used GEE because this method allows for the consideration of data correlation in the repeated observations on the same subjects (within-subject correlation).

The model was fitted using different correlation structures: independence, autoregressive of order 1 (AR 1), exchangeable, and unstructured. Based on the Akaike Information Criterion (AIC), the models fitted better under the independence correlation matrix. P-values less than 5% were considered statistically significant.

All analyses were performed in R version 4.2.1 (Bivand *et al*., 2022). The package geepack version 1.3.9 (Halekoh *et al*., 2006) was used to carry out the regression analyses. The relationship between the study variables was modeled using the following code: geeglm (dependent variable ~ shadow + time + shadow * time, data = data, id = id, family = Gaussian (link = identity), corstr = independence).

Results and Discussion

The interactions of the shadow with multiple external and internal factors (Koutouleas *et al*., 2022) justify the use of statistical tools that allow for the characterization of the fluctuation of phenological variables. We take the median as a reference point in a Boxplot analysis (Figure 1) and compare the sources of variation (S, T, and S x T) with Control S2. We use a GEE model that allows for contrasting them while considering the correlation originating from repeated observations over time (Figure 2).

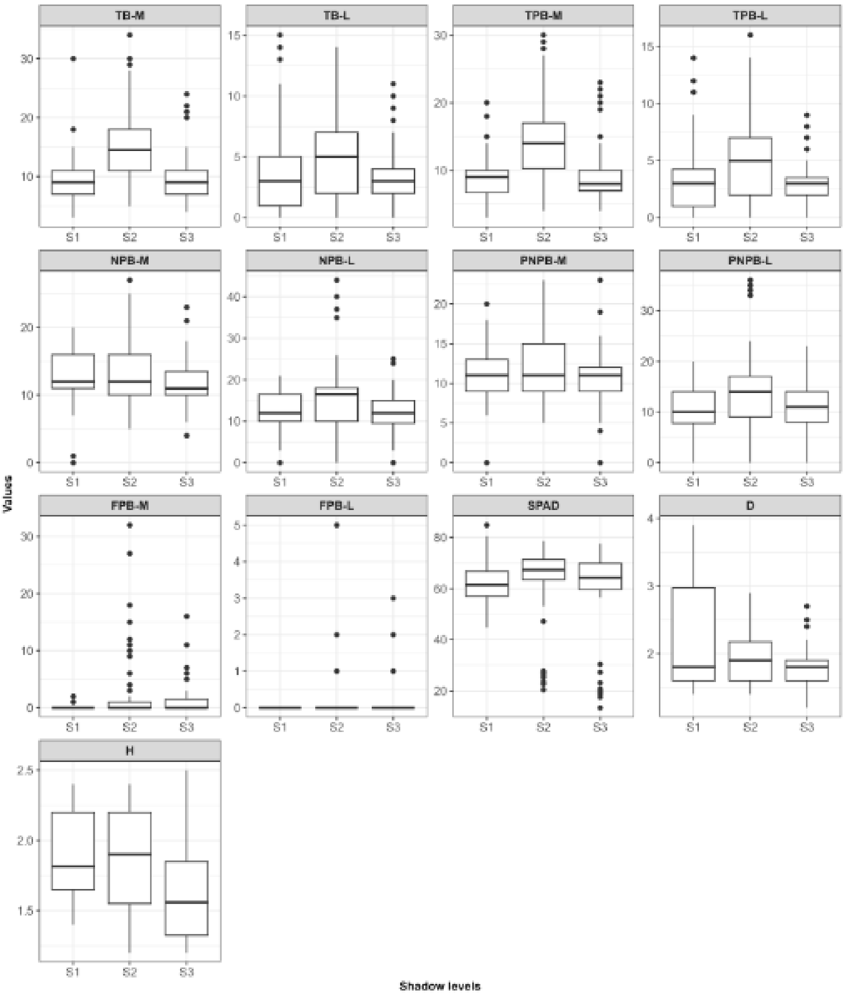

Figure 1: *Effects of shadow levels (S1, S2, and S3) on phenological variables of Arabica coffee trees (Coffea arabica* L.*) measured fortnightly, first trimester of postharvest development. Total branches in the middle third (TB-M) and lower third (TB-L); Total productive branches in the middle third (TPB-M) and lower third (TPB-L); Nodes of a productive branch in the middle third (NPB-M) and lower third (NPB-L); Productive nodes of a productive branch in the middle third (PNPB-M) and lower third (PNPB-L); Fruits (or flowers) in productive branches of the middle third (FPB-M) and lower third (FPB-L); Relative amount of chlorophyll measured in SPAD units (C); Diameter (cm) of the stem of the coffee tree at 15 cm from the ground (D); Height (m) of the coffee tree (H).*

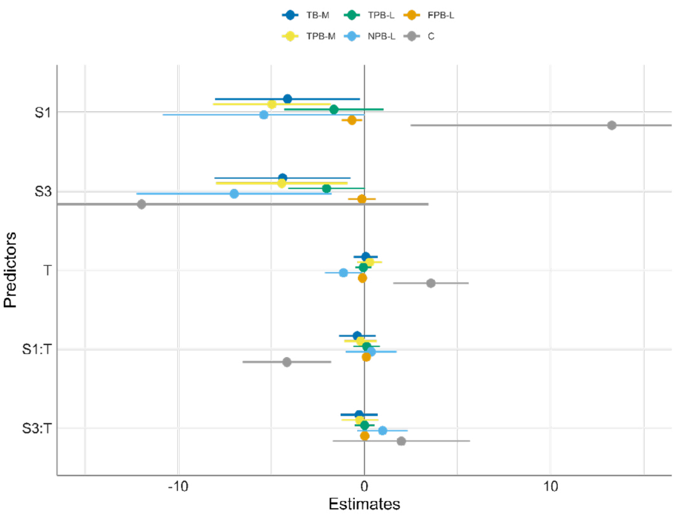

Figure 2: *Model results with significant estimates. Effects of high (S3) and low (S1) shadow levels, time (T) and S x T interaction on dependent variables, compared to a shadow level as a control (S2=0). Total branches in the middle third (TB-M) and lower third (TB-L); Total productive branches in the middle third (TPB-M) and lower third (TPB-L); Nodes of a productive branch in the middle third (NPB-M) and lower third (NPB-L); Productive nodes of a productive branch in the middle third (PNPB-M) and lower third (PNPB-L); Fruits (or flowers) in productive branches of the middle third (FPB-M) and lower third (FPB-L); Relative amount of chlorophyll measured in SPAD units (C); Diameter (cm) of the stem of the coffee tree at 15 cm from the ground (D); Height (m) of the coffee tree (H).*

In the first analysis, it is possible to delve into the trends suggested by the distribution of the variables to find the most biologically relevant effect of the shadow on the development of coffee trees, regardless of strict statistical validation. On the other hand, in the second analysis, we emphasize significant effects to establish a hierarchy of the effects of all sources of variation, including temporal and its interaction with shadow levels. With these two approaches, we intend to achieve complementarity that reduces the bias of experimental error.

The results are organized in three parts, considering the logic used in their discussion: (1) Growth of branches, flowers, and fruits, (2) Chlorophyll content (SPAD units), and (3) Coffee plant height and stem diameter.

Growth of branches, flowers, and fruits

In the experiment, the tree canopy irregularly distributed in the study area originates places of fluctuating shade ranging from 70% to 0%, with three levels: S1 (0-30%), S2 (31-50%), and S3 (51-70%) under treatment S. In Figure 1, the phenological variables related to the vegetative development of coffee trees (TB-M, TB-L, TPB-M, TPB-L) showed higher central (median) values in S2 compared to S1 and S3, as well as their dispersion. This was especially true in the middle stratum (TB-M and TPB-M). Overall, these results are aligned with those reported by Campanha *et al.* (2004), DaMatta (2004), and are linked to the photosynthetic activity associated with the greater intensity of incident solar radiation.

The production of precursor nutrients for vegetative growth in the plant can be directly related to radiation intensity (Castillo and López, 1966). These authors found a strong relationship between radiation intensity and flowering and vegetative growth measured by the number of leaves. The increase in light intensity is proportional to foliage growth and flowering, although that relationship is not linear. The growth model described fits a sigmoid, with the typical self-acceleration phase with exponential increases in growth velocity, followed by another decelerated phase, conditioned by the limiting factors of the environment.

This explains the existence of non-uniform effects of shade, with vegetative growth, flowering and grain formation stimulated by the increase in incident radiation, without exceeding the limits of the inflectionpoint (tipping point) from which the decrease in growth rate begins. This is congruent with the greater vegetative development in S2 (see TB M, TB L, TPB M, TPB L in Figure 1), but not with the absence of effects on flowering and fruiting (see FPB M and FPB L in Figure 1). In fact, in our results, flowering and fruiting are not affected by the level of shade, nor are their precursors, i.e., nodes per productive branch (NPB M and NPB L) and productive nodes per productive branch (PNPB M and PNPB L).

It is generally accepted that the higher intensity of radiation accelerates all physiological processes associated with the synthesis of metabolites that lead to early flowering, according to numerous authors (DaMatta, 2004; dos Santos Freire Ricci *et al.*, 2006; Jaramillo-Botero *et al.*, 2010; Morais *et al.*, 2008), which explains the moderating effect of shade on coffee biennially in AFS (Lin, 2007; Morais *et al.*, 2006, 2009; Heverly *et al.*, 2003). These effects did not occur in our case, which could be explained by the short observation period (90 days).

There is a consensus that attributes these adaptive changes in shade to a much longer physiological process (Alemu, 2015; Cannell, 1985; Chaves *et al.*, 2008; DaMatta, 2004; Smith and Whitelam, 1997). The starting point to explain the seasonal lag in production lies in the phenology of the plant and in the role of water stress and thermal regime on the distribution of carbon in the different organs of the coffee tree. Thus, the seasonal asymmetry in the thermal regime and rainfall determines a sequence that only closes in biannual cycles, with the succession of periods in which these microclimatic variables alternate seasonally, conditioning the vegetative development and, later, that of productive nodes and fruits (Vaast *et al.*, 2006). On the other hand, flowering would be induced by the rains that follow the dry period (DaMatta *et al.*, 2007) and intensified by light intensity (Franck and Vaast, 2009).

The results of the GEE analysis in Figure 2, to estimate the effect of shade on the different dependent variables, reiterate the picture described above in terms of vegetative development and flowering and fruiting: S2 (31-50%) compared to S1 (0-30%) and S3 (51-70%) favors vegetative development (TP-M, TP-L, TPB-M, and TPB-L), but not that of flowering and fruiting (FPB-M, FPB-L) and their immediate precursors (NPB-M, NPB-L, PNPB-M, and PNPB-L). This is in line with the above argument related to the short period of time in which the six biweekly measurements that completed the 90 days of the experimental period took place.

Chlorophyll content (SPAD units)

Comparison of the central values of C in Figure 1 suggests that the distribution is approximately normal, with relatively homogeneous central values, closer in S2 and S3, but higher than in S1, especially in S2. A first approximation suggests that chlorophyll measured in SPAD units tends to decrease in environments with less shade, and vice versa. These results are consistent with the consensus that identifies coffee as a plant phylogenetically adapted to shade, with homeostatic mechanisms to avoid the detrimental effects of excess radiation on photosynthesis (Chaves *et al.*, 2008; Moraes *et al.*, 2010).

When regression analysis with the GEE model is used in Figure 2, including time (T) and its interaction with shade level (S), it was found that the effect of S is modified by T, and therefore the results should not be examined in isolation. It was found that the duration of treatment (T) modified the effect of S1, finding a greater decrease in C in coffee trees with less shade, increasing the time from the first to the sixth observation at 90 days (Figure 2). However, although the opposite could not be proven, that is, the increase of C in the S3 treatment over time (T), significant differences were found between S1 and S3 in which the effects on chlorophyll content (C) are polarized. This proves the photoinhibition induced by a high incident radiation in S1 (decrease of C with time T) and compensatory mechanisms to increase the net efficiency of photosynthesis in S3 (increase of C with time T), all in accordance of the physiological mechanism of the response to shade (Castillo and López, 1966; Smith and Whitelam, 1997). These results are consistent with the consensus that identifies coffee as a plant phylogenetically adapted to shade, with homeostatic mechanisms to avoid the detrimental effects of excess radiation on photosynthesis (Chaves *et al.*, 2008; Moraes *et al.*, 2010).

Although the inverse relationship between shade and chlorophyll content has been reported by numerous authors (Bote *et al.*, 2018; Bote and Struik, 2011; Encalada-Córdova *et al.*, 2016; Hollies, 1967; Khan *et al.*, 2000; Mariño, 2014; Zhang *et al.*, 2019), further studies are needed to understand the physiology underlying the effect of shade on coffee (Piato *et al.*, 2020). In our study, the shade levels considered optimal (S2, 31-50%) coincide with those associated with a significant increase in net photosynthetic rate and light use efficiency by (Zhang *et al.*, 2019). It is appropriate to mention the possible relationship between the increase in chlorophyll content as an adaptation to the decrease in absorption of photosynthetically active radiation in the shade.

According to Charbonnier *et al.* (2017), in response to this reduction, the coffee plant increases its light use efficiency, thus maintaining its net primary productivity. It is interesting to note that in this type of studies on the complex physiological mechanisms involved in the adaptive response to changes in radiation intensity in coffee agroforestry systems, the experimental periods are usually longer than two years, which contrasts with the short period of three months in our work. However, even in this short time, the average C in all treatments increased significantly as a function of T (Figure 2).

We found that the duration (T) of treatment modified the effect of S1, finding a greater decrease in C in coffee trees with less shade, increasing the time from the first to the sixth observation at 90 days (Figure 2). Although the opposite could not be proven, the increase of C in the S3 treatment over time (T) significant differences were found between S1 and S3 in which the effects on chlorophyll content (C) are polarized. This evidences the photoinhibition induced by a high radiation incident in S1 (decrease of C with time T), and compensatory mechanisms to raise the net efficiency of photosynthesis in S3 (an increase of C with time T), all in correspondence with the referred bimodal character of the physiological mechanism of the response to the shade(Castillo and López, 1966; Smith and Whitelam, 1997).

Coffee plant height and stem diameter

As reported in the overview of the analyses, only statistically significant effects were presented in Figure 2, so we excluded Diameter (D) and Height (H), which did not differ between treatments (Figure 2). Furthermore, the variation around the median of these indicators (Figure 1) did not reveal any truly relevant behavior. These results contrast with those of Bote *et al*. (2018) and are also inferred from the studies of Morais *et al*. (2006) and Campanha *et al*. (2004). The former found an elongation of the stem, while the latter observed a reduction in the growth of plagiotropic branches, both representing typical adaptive responses of coffee trees. These responses are part of the photoinhibition syndrome already mentioned (Smith and Whitelam, 1997). However, all these studies had an experimental period extending up to 2 years, whereas our work only spanned 90 days. This shorter duration was clearly insufficient to demonstrate this effect.

Conclusions and Recommendations

Using simulations of the shadow cast by trees in the Agroforestry System (AFS), three levels (treatments) of shade were identified: S1-High (51-70%), S2-Pattern (31-50%), and S3-Low (0-30%). Biweekly measurements of early growth indicators and estimated chlorophyll content were measured using SPAD units in randomly chosen leaves. The results confirm the significant influence of light on the development of plagiotropic branches and the decrease in chlorophyll concentration. This decrease indicates a photo-inhibition due to excessive radiation levels even in the early stages of plant development. The performance observed aligns with the homeostatic mechanism of Arabica coffee, which has adapted to environments with low radiation intensity. These findings provide a promising signal to promote the use of AFS in areas classified as “suboptimal” due to their low altitude. Despite the inclusion of time as a variable in the experimental design, it is necessary to extend the experimental period to evaluate other impacts of tree shadows, such as biennially, grain quality, and the organoleptic properties of ready-to-drink coffee. Furthermore, it is important to deepen our understanding of the most suitable species for structuring the tree canopy in the AFS

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Novelty Statement

The results confirm the significant influence of light on the development of plagiotropic branches and the decrease in chlorophyll concentration (SPAD Units), and consequently, a photo-inhibition caused by excessive radiation levels, even in early stages of plant development. This behavior is consistent with the homeostatic mechanism of Arabica coffee originated in its adaptive response to environments with low radiation intensity and constitutes a promising signal to promote the use of AFS in areas classified as suboptimal due to the low altitude at which they develop.

Author’s Contribution

Julio Adolfo Corzo-Bacallao: Conceived and designed. Supervision and drafting of the manuscript and preparation of the final draft of the manuscript.
Carlos Alfredo Salas-Macías: Conceived and designed. Contributed data or analysis tools. Review and editing.

Osvaldo Fonseca-Rodríguez: Performed the analysis, review and editing.

Felipe R. Garcés-Fiallos: Drafting of the manuscript and preparation of the final draft of the manuscript. Review and editing.

Erika Isabel Alcívar-Muñoz and Henry Fabricio Baque-Loor: Field research and data collection.

All the authors of this work have read and approved the final version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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