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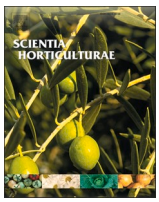

Varying fruit loads modified leaf nutritional status, photosynthetic performance, and bean biochemical composition of coffee trees

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ABSTRACT

Changes in the intensity of the sink organs in coffee trees can alter photosynthetic activity and accumulation of nutrients at the foliar level and increase the magnitude of malformed fruits, as well as affecting bean biochemical composition. Therefore, the objective of this study was to evaluate the effect of different intensities of fruits load on photosynthetic performance, nutritional status, yield, and bean biochemical composition of *Coffea arabica* trees established under full sun field-grown conditions. The evaluations were carried out on three-year-old "Cenicafé 1″ variety trees after their establishment in the field. Nine treatments (fruit load), of 20, 30, 40, 50, 60, 70, 80, 90, and 100 % according to total crop load, were applied at the whole-plant level. Gas exchange, nutrient composition, chlorophylls, and soluble sugars were determined in the leaves. Yield components, sugars, organic acids, and alkaloid concentration were determined in the fruits and beans. With a high fruit load, significant leaf nutrient concentration changes were detected, with deficiencies in nitrogen, potassium, sulphur and copper. Furthermore, significant decreases in the chlorophyll content and stability, as well as in the total soluble sugars at the leaf level, were reported. On the other hand, it is evident that the production per plant was higher, although the number of malformed fruits increased with elevated fruit load. Finally, accumulation of sugars, organic acids, and alkaloids in the bean was modified as a function of the increase in fruit load. These results indicate that, in coffee "Cenicafé 1″ variety trees with high fruit load, the nutrient concentration decreased to critical levels considered to nutritional deficiencies, which modified photosynthetic performance, number of malformed fruits, and bean biochemical composition.

1. Introduction

A highly coordinated source-sink relationship for the supply of photosynthetic products and demand of assimilates is a key regulator in the foliar photosynthetic activity of plants (Cheng et al., 2021; Wang et al., 2022). The interactions between source photosynthetic activity and sink strength caused by increased fruit load for *Coffea arabica* L trees have been well documented (Chaves et al., 2012; Avila et al., 2020; Almeida et al., 2021). It has been shown that elevated fruit load contributes to higher leaf photosynthetic rates, associated with increased stomatal conductance (DaMatta et al., 2008; Unigarro et al., 2022). However, low sink demand has been reported to have negative impacts on photosynthesis resulting from diffusive (stomatal and mesophyll) and biochemical limitations that cause restrictions in CO_2 assimilation, low RuBisCO activity, and high concentrations of carbohydrates in the source leaves, which can promote negative feedback on photosynthesis (Franck et al., 2006; Almeida et al., 2021).

In coffee trees with a high source-sink ratio, it has been found that reductions in leaf photosynthetic rates are not associated with down-regulation of direct feedback mechanisms but rather with environmental stimuli or plant growth characteristics (Avila et al., 2020; Almeida et al., 2021; Toro-Herrera et al., 2023). For example, under conditions of low fruit load, the vegetative growth that coincides with the reproductive phase may act as a sink with adequate strength to regulate the soluble sugars and starch accumulation in the leaves (DaMatta et al., 2008). Likewise, it has been postulated that the

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phenological stages of fruit development play a crucial role in the leaf photosynthetic performance since, at the fruit filling stage, leaf photosynthetic activity has been found to significantly increase compared to the fruit formation and ripening stages (Vaast et al., 2005; Ávila et al., 2020; Unigarro et al., 2022).

The low source-sink regulation in *C. arabica* has been reported to lead to limitations in nitrogen metabolism during the fruit linear growth or endosperm-filling phase (Bote and Vos, 2016; Avila et al., 2020). This is because coffee fruits are considered priority sinks, and require large quantities of carbohydrates and nutrients, in particular, nitrogen and potassium, which can eventually lead to deficiencies of these nutrients in the source leaves (Chaves et al., 2012; Bote and Vos, 2016). Under nitrogen deficiency conditions, photosynthesis is impaired by the degradation of the photosynthetic pigment pooling caused by excess energy, which can trigger more severe oxidative damage at the cellular level. On the other hand, potassium deficiency has been related to the low activity of enzymes linked to sugar metabolism in source leaves, which affects the production and translocation of photoassimilates to the sink organs (Pompelli et al., 2010; de Bang et al., 2021, Souza et al., 2020).

It has also been shown that the concentrations of alkaloids and sugars are reduced in *C. arabica* beans by limitations in the availability of nutrients such as nitrogen and potassium (Vinecky et al., 2017; Ahmed et al., 2021). Moreover, high fruit load can alter the accumulation patterns of bean metabolites (Chaves et al., 2012; Cambou et al., 2021). This result has been associated with the strong competitions for carbohydrates promoted among the fruits (Vaast et al., 2005; Läderach et al., 2011; Bote and Vos, 2017). Vaast et al. (2006) report that the caffeine and trigonelline contents of the bean are reduced with elevated fruit load, whilst Läderach et al. (2011) and Bote and Vos. (2017) found that sensorial attributes of the beverage are altered by high fruit load. However, there is little information on the effect of fruit load on the bean biochemical composition of field-grown coffee trees established in full sun conditions (Läderach et al., 2011).

The fruit formation of coffee in Colombia lasts from 32 to 34 weeks after flowering and presents four key phenological stages related to the dry mass accumulation of the fruit, described according to the BBCH scale for *Coffea* spp (Arcila-Pulgarín et al., 2002). The slow growth phase-BBCH71 which occurs approximately in the 4 to 8 weeks after flowering, the linear growth phase or fruit filling (BBCH73), which has been estimated to take place between 9 and 28 weeks after flowering, and this phase ends with the physiological maturity of the fruits-BBCH79 (Ocampo et al., 2010). Finally, the stabilization phase, which coincides with the beginning of fruit ripening until harvest, lasts between 32 and 34 weeks after flowering (Sanz-Uribe et al., 2018; Osorio Pérez et al., 2023a).

In this study, we test the following hypotheses: (*i*) the manipulation of different fruit load intensities will reveal that the leaf photosynthetic activity is related to fruit development stages; (*ii*) the low autonomy in high fruit load branches will eventually lead to nutrients deficiencies during endosperm-filling stage; and (*iii*) in coffee trees, manipulation of fruit load levels will reveal if there are changes or alterations in the accumulation of bean metabolites caused by strong sink strength of coffee berries. Therefore, our objective was to evaluate the effect of different fruit load intensities on the photosynthetic performance, nutritional status, yields, and bean biochemical composition in *C. arabica* "Cenicafé 1″ variety trees established under full sun field-grown conditions in the coffee zone of central Colombia. This information is necessary in order to understand the consequences of the high fruit load commonly reported in equatorial or tropical regions on the physiological performance of coffee plants during years of high production.

2. Material and methods

2.1. Study area, growth conditions and treatments description

This experiment was carried out with three-year-old "Cenicafé 1″ variety trees established under full sun field-grown conditions at the Naranjal Experimental Station of the National Coffee Research Center (Cenicafé), located in Chinchiná, Caldas (04° 58′ N; 75° 39′ W), at 1383 m a.s.l. The average environmental conditions were determined from April to November 2022 in relation to the phenological stages of fruit development-BBCH: PAR of 785.17 μmol m^2 s^{-1}, sunlight of 3.47 h d^{-1}, temperature of 20.74 °C, VPD of 2.19 KPa (Fig.S1) and accumulated rainfall of 2088 mm (Table. S2). The selection of this tree age was based on the fact that it is one of the most abundant annual harvests of the productive cycle in Colombia (Rendón, 2020). The trees were cultivated in full sunlight and were planted at a spacing of 1.4 m x 1.0 m, with one orthotropic branch or ramification. In accordance with agronomic practices, they received amendments and fertilizer applications based on a soil analysis (Table S3). These consisted of lime (135 g $plant^{-1}$), urea: 591 kg ha^{-1} (82 g $plant^{-1}$), MAP: 80 kg ha^{-1} (11 g $plant^{-1}$), KCl: 490 kg ha^{-1} (70 g $plant^{-1}$) and Kieserite: 210 kg ha^{-1} (30 g $plant^{-1}$), which were applied two times per year (February and August) and in equal proportions for all plants according to recommendations (Sadeghian, 2022).

The trees were subjected to nine treatments applied at a whole-plant level and to each branch, at the time when trees bore pinhead fruits at the BBCH71 phenological stage (slow fruit growth), as reported by Arcila-Pulgarín et al. (2002) on April 18, 2022. Prior to the application of the treatments, the trees with the highest fruit load in the function crop initial load, which presented around 1910 fruits $plant^{-1}$ (T1: 100 % fruit load) were selected. Subsequently, the other treatments were assigned as follows: T2: 90 % fruit load (1719 fruits $plant^{-1}$), T3: 80 % (1528 fruits $plant^{-1}$), T4: 70% (1337 fruits $plant^{-1}$), T5: 60 % (1146 fruits $plant^{-1}$), T6: 50 % (955 fruits $plant^{-1}$), T7: 40 % (764 fruits $plant^{-1}$), T8: 30 % (573 fruits $plant^{-1}$) and T9: 20 % fruit load (382 fruits $plant^{-1}$). To maintain uniformity within each treatment, fruit thinning was performed on individual branches whenever the fruit load exceeded the specified percentage. In total, 54 trees were selected based on their uniformity with regard to height (175.82 ± 0.76 cm, ±: standard error-SE) and number of productive plagiotropic branches (39 ± 2.50). For measurement purposes, four plagiotropic branches located in the central region of the canopy, which is typically the most productive zone, were chosen from each tree (Bote and Vos, 2016). These selected branches displayed a range of 20–24 fully expanded leaves, between 32 and 143 fruits per branch and leaf area-to-fruit ratio from 25.17 to 10.51 cm^2.

2.2. Leaf physiological measurements

2.2.1. Gas exchange

Measurements of gas exchange net photosynthetic rate-*A*, stomatal conductance-g_s, intracellular carbon concentration-C_i, transpiration-*E*, and extrinsic water efficiency use-WUE_e (A/E) were performed with an infrared CO_2 / H_2O gas analyzer (CIRAS-3, PP Systems, Amesbury, MA, USA). The measurements were carried out from 8h30 to 10h30 in the middle part of the adaxial surface on the leaf blade on eight fully developed leaves (for each treatment), selected from the third or fourth pair of leaves from the apex of a plagiotropic branch (León-Burgos et al., 2022). The measurements were conducted with a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1}, 400 ppm CO_2, leaf temperature at 25 °C, relative humidity between 50 and 70 %, and maintaining the natural leaf angle. In order to describe the relationship of gas exchanges parameters with the fruit development, all the measurements were carried out at four key phenological stages: slow growth phase- BBCH71, linear or accelerated growth phase-BBCH73 until physiological maturity-BBCH79, and stabilization phase with ripening at harvest time

-BBCH88 (Ávila et al., 2020).

2.2.2. Determination of chlorophylls and soluble sugars contents

The sample processing was carried out with fully developed fresh leaves selected from the third or fourth pair of leaves from the apex of the plagiotropic branch at the phenological stage of fruit development BBCH79 (physiological maturity). For this, four samples were taken for each treatment. The leaves were bevel cut from the petiole using a knife and stored in zip-lock bags containing wet paper towels to prevent leaf dehydration. In the laboratory, for the determination of chlorophylls a and b, the leaves were cleaned with deionized water, dried at room temperature, and then stored in labeled paper bags and lyophilized for 48 h (Kumar et al., 2015). Subsequently, samples were ground with liquid nitrogen and frozen at −75 °C.

The equation [1] proposed by Lichtenthaler (1987) was used to determine the content of total chlorophyll (*TChl*). Dried leaf tissue samples of 500 mg were used and homogenized in 5 mL of 95 % ethanol (v/v) and then subjected to ultrasound for 30 min (08893–21, Cole Parmer, USA). Subsequently, samples were centrifuged (21r, Beckman Allegra, Germany) for 10 min at 4000 rpm, and the supernatant was removed. Ethanol was added to dilute the supernatant to a final volume of 25 mL. A spectrophotometer (DU600, Beckman, USA) was used to determine the content of TChl at 664 and 648 nm.

$$TChl\ \left(mg\ g^{-1}\ DM\right) = 5.24\ Abs\ 664 + 22.24\ Abs\ 648.6 \quad (1)$$

Where *TChl* is the content of total chlorophylls and *Abs* absorbance values at 664 and 648 nm. To calculate the chlorophyll stability index-*CSI*, a control sample was taken from leaves extracted from vegetative phase branches of the same trees that were evaluated at different fruit load intensities. Equation [2] was used to determine *CSI* (Mohan et al., 2000).

$$CSI\ (\%) = \frac{TChl\ samples\ with\ fruit\ loads}{TChl\ control\ samples} * 100 \quad (2)$$

The content of total soluble sugars-TSS was measured in the same sample taken for the determination of chlorophylls. The samples were diluted with 0.5 mL of phenol and 2.5 mL of H_2SO_4. Then, the sample was vortexed for 1 min and left to settle for 30 min. Subsequently, it was centrifuged for 2 min at 9000 rpm (Microfuge 20, Beckman Coulter, Nyon, Switzerland). A spectrophotometer (DU600, Beckman, Fullerton, CA, USA) was used to determine the content of soluble sugars at 645 nm (Roby and White, 1987).

2.3. Leaf nutrients composition

The samples were taken based on the methodology described by Carrillo et al. (1994) during the phenological stage of fruit development-BBCH79 (physiological maturity). For analysis, three trees (12 leaves per tree) were selected at random for each treatment. The leaves were selected from the third or fourth pair of leaves from the apex of the plagiotropic branches located in the middle part of the canopy. Then, the collected material was dried in an oven at 65 °C for 72 h. Subsequently, the dried leaves were ground in a Foss Cyclotec 1093 device with a 0.5 mm mesh and stored in a hermetic vial.

The concentrations of the nutrients P, K, Mg, Fe, Mn, Zn, and Cu were determined via dry combustion (incineration in a muffle at 475 °C for five h). The plant material was dissolved in a 17 % HCl acid solution (v/v). The determination of K, Mg, Fe, Mn, Zn, and Cu was performed by atomic absorption spectrometry (55AA Spectrometer, Agilent technologies, Malaysia) and P with UV/Vis spectrometry (Lambda 365 UV/Vis, PerkinElmer, Korea) through the vanadate-molybdate and azomethine-H methods. The N content was measured by the Kjeldahl semimicro method and S content by incineration of $Mg(NO_3)_2$ with the turbidimetric method (Carrillo et al., 1994).

2.4. Yield components

The number of fruits, percentage of malformed fruits, weight and equatorial diameter of the individual fruits, and the annual production were measured at the phenological stage of fruit development-BBCH88 (ripening). The number of fruits was determined with direct counts on the branches. The production was quantified by the amount of coffee berries per plant (g) using a 100 g precision dynamometer. For harvesting, which was carried out at intervals of 18 d from May to December 2022 only entirely red fruits were selected.

To determine the fruit weights, 30 fruits were selected randomly from the harvested mass by each tree, and these were weighed on 0.01 g precision balance. These measurements were made during the main harvest events reported for the study area between September 27 and November 3, 2022 (Rendón, 2020). Thus a total of 540 fruits were obtained per treatment. Subsequently, the equatorial diameters of the same fruits that were taken for the measurement described above were measured with a digital Vernier caliper (500–196–30B, Mitutoyo, Joinville, Brazil). The number of malformed fruits was obtained with a sample size of 100 coffee berries for each tree. The sample was selected at random from the harvested mass, using only healthy fruits. Subsequently, the fruits were submerged in a container with 5 L of water, and the number of floating fruits was recorded. For the estimated percentage of malformed fruits, equation [3] was used. These measurements were made during the main harvest events reported for the study area.

$$Malformed\ fruits\ (\%) = \left[\frac{Floating\ fruits}{Total\ fruit\ samples}\right] x\ 100 \quad (3)$$

2.5. Quantification of bean biochemical composition

2.5.1. Sample processing

The coffee beans were subjected to the standard practices for wet processing recommended by Peñuela-Martínez et al. (2022). This was done in the order as follows: *i*) selective harvesting was performed to avoid the presence in the harvested mass of more than 2.5 % of green grains; *ii*) pulping was carried out within 6 h after harvest: and *iii*) natural fermentation process was controlled with Fermaestro® to determine the ending time of coffee mucilage fermentation. Then, fruits were dried until they reached a humidity content between 10 and 11.5 %. Finally, the samples were threshed, and healthy beans were selected.

In order to conduct measurements, 80 g samples of beans were taken with six replicates for each treatment. Each sample was cryogenically ground with liquid nitrogen and stored at −80 °C. All biochemical compounds were identified by comparison of retention times with standards and quantified using calibration curves with an external standard according to the compound of interest (Osorio Pérez et al., 2023a; 2023b).

2.5.2. Soluble sugars

The concentrations of sucrose, glucose, and fructose were determined. Samples of 0.10 g were used and diluted in 10 ml distilled water. Then, this was vortexed for 20 s and subjected to a water bath at 80 °C for 30 min. Subsequently, each sample was 0.22 μm filtered and deposited in 2 mL vials. For the separation and quantification of sugars, an HPLC system (2690, Waters Alliance, Milford, MA, USA) coupled to a 2414 refractive index detector was used, and the process was performed with a Sugar Pack I column (6.5 × 300 mm, 10 μm, Waters, Milford, MA, USA) at 85 °C with deionized water at a flow rate of 0.5 mL min^{-1} (Osorio Pérez et al., 2023a).

2.5.3. Organic acids

Five organic acids associated with the coffee sensory quality according to Osorio et al. (2023b), were evaluated, namely acetic acid, malic acid, citric acid, quinic acid, and lactic acid. For the determination of these compounds, 0.80 g samples were used and homogenized in 25

mL distilled water at 96 °C temperature and vortexed for 5 min. Then, each sample was 0.22 μm filtered and deposited in 2 mL vials. The organic acids were determined using a Waters 600E HPLC system coupled to a diode array detector (DAD-996), with a Hi-Plex H column (Agilent, 7.7 × 300 mm, 8 μm) at a temperature of 50 °C and a flow of 0.5 mL min^{-1} with acidified deionized water (0.01 M H_2SO_4) and detection at 210 nm.

2.5.4. Alkaloids

Samples of 0.20 g were diluted in 40 mL distilled water and vortexed for 1 min. The extraction was carried out by refluxing with 90 °C water for 25 min. Ultrapure water was added to dilute to a final volume of 50 mL. Then, each sample was 0.22 μm filtered and deposited in 2 mL vials. The alkaloids caffeine and trigonelline were determined. For this, a Waters 600E HPLC system coupled to a diode array detector (DAD-996) was used with a Symmetry C18 column (Waters, 4.6 mm x 250 mm, 100 Å, 5 μm) at 35 °C with a flow rate of 1.0 mL min^{-1} in a mixture of water, methanol, and acetic acid (59:40:1 v/v/v) and detection at 273 nm.

2.6. Experimental design and statistical analysis

A completely randomized experimental design with nine treatments was used for data analysis. The experimental unit was considered a coffee plant, with one experimental unit per repetition and six repetitions per treatment ($n = 6$). To evaluate the effect of the phenological stages of fruit development-BBCH on leaf gas exchange, an analysis of variance of repeated measures was performed using the F statistic ($p < 0.05$). Subsequently, a post hoc test for the comparison was applied using the Tukey-Kramer test ($p < 0.05$). For the measurements of nutritional status, chlorophylls, and bean biochemical composition, an

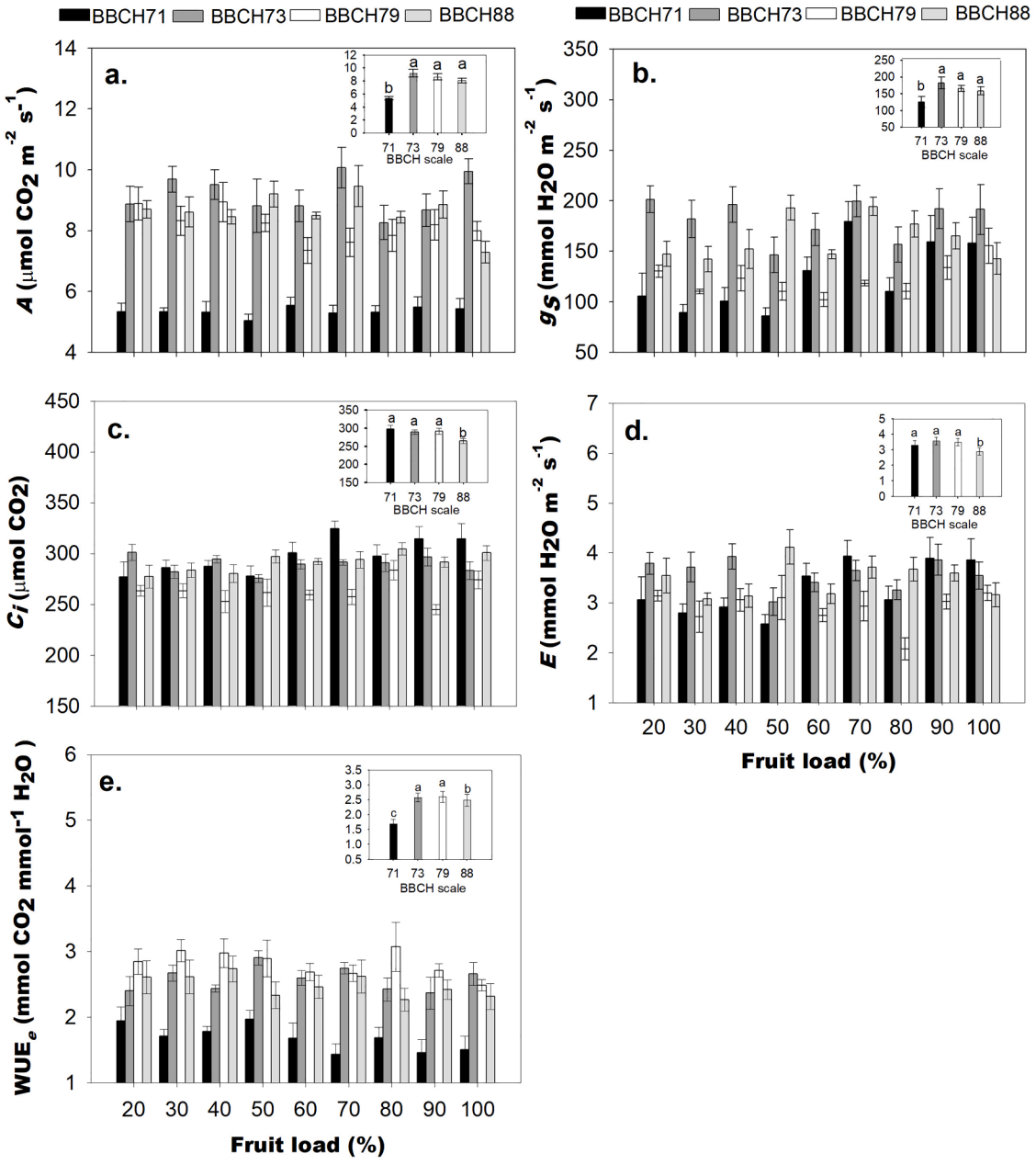

Fig. 1. The effect of the fruit load on net photosynthetic rate-*A* (a), stomatal conductance-*gs* (b), internal carbon concentration-*Ci* (c), transpiration rate-*E* (d), and water use efficiency extrinsic -*WUEe* (e) according to BBCH scale for fruits development. Each data is the mean ($n = 8$) ± SE. In the insets, mean values of *A, gs, Ci, E* y *WUEe* according to the BBCH Scale for fruit development are shown. The different letters denote significant differences among means within each BBCH scale for fruit development according to the Tukey test ($p < 0.05$).

analysis of variance (ANOVA, $p < 0.05$) was used after fulfilling the assumptions of normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test). Then, the comparison of the means between the treatments was carried out with the Tukey test ($p < 0.05$). For the content of leaf soluble sugars-*TSS*, a linear regression analysis was used, which was fitted with the following statistical criteria: coefficient of determination-R^2, residual standard error, and the significance of the parameters of the equation according to the t-Student test ($p < 0.05$).

Pearson correlation analyses were performed to evaluate the relationships between fruit load and the leaf concentrations of N, K, S, Cu, *TChl* and *TSS* using the Corrplot statistical package version 0.92 (Wei y Simko, 2021). Finally, to demonstrate the relationship between bean biochemical attributes and fruit load, a heatmap representation with hierarchical grouping was made with the gplots package version 3.1.3 (Warnes et al., 2022). All statistical analyses were performed with R software version 4.0.4 using Stats and Agricolae packages version 1.3–7 (Mendiburu, 2023; Development Core Team, 2019), and for graphics, Sigmaplot 10.0 (Inpixon, Systat Software Inc., USA) was used.

3. Results

3.1. Interplay between leaf gas exchange and varying fruit load

The measurements of foliar gas exchange did not show significant variations with the fruit load. In contrast, there were statistical differences in the phenological stages of fruit development-BBCH. For the slow stage -BBCH71, the lowest average values of *A* were recorded, at 5.35 μmol of $CO_2 m^2 s^{-1}$. On the other hand, at the accelerated or linear stage -BBCH73, increases of 71 % were found, with the highest value during the full fruit development: *A* 10.08 μmol of $CO_2 m^2 s^{-1}$. Likewise, during the BBCH79 and BBCH88 stages, increases and significant differences were reported in photosynthetic rates with respect to BBCH71 stage (Fig. 1a). This same result was recorded for g_s, with statistical differences and higher values of g_s, at 169.83 mmol $m^2 s^{-1}$, reported for

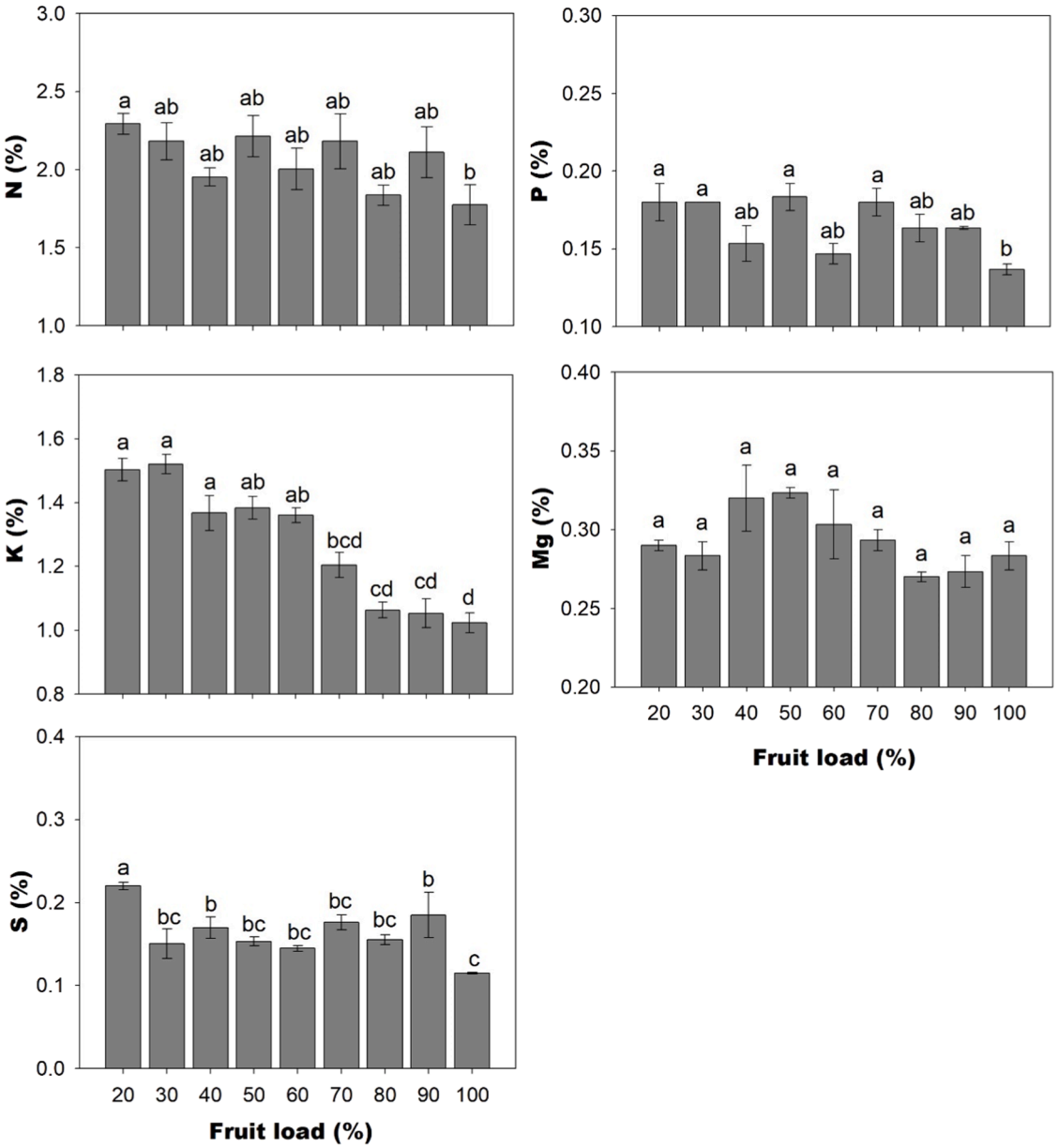

Fig. 2. Effect of the fruit load on leaf concentration of Nitrogen-N- (a), Phosphorous-P (b), Potassium-K (c), Magnesium-Mg (d), and Sulphur-S (e). These measurements were made in fully developed leaves. Each data is the mean ($n = 3$) ± SE. The different letters denote significant differences among means within treatments according to the Tukey test ($p < 0.05$).

stages of fruit development BBCH73, BBCH79 and BBCH88, compared to the BBCH71 stage with lower average values of g_s at 124.53 mmol m^2 s^{-1} (Fig. 1b).

C_i and E showed variations at the end stage of fruit development. At the BBCH71, BBCH73 and BBCH79 stages, average values of C_i of 292.77 µmol and E 3.45 mmol m^2 s^{-1} were reported, which show statistical differences compared to the BBCH88 stage, where lower values for C_i of 264.97 µmol and E 2.89 mmol m^2 s^{-1} were found (Fig. 1c and d). On the other hand, WUE_e showed the highest average values (2.53 mmol CO_2 $mmol^{-1}$ H_2O) at the BBCH73 and BBCH79 stages, with significantly differences in relation to the BBCH88 and BBCH71 stages. However, the lowest average values of WUE_e, of 1.69 mmol CO_2 $mmol^{-1}$ H_2O, were reported at the slow stage of fruit growth -BBCH71 (Fig. 1e).

3.2. Changes at the leaf level of nutritional status, content of chlorophylls, and soluble sugar in relation to fruit load

The fruit load promoted alterations in the leaf nutrient concentration of coffee plants. For lower fruit loads (20, 30, and 40 %), the concentration of nitrogen-N (2.14 % on average), phosphorus-P (0.18 %), and potassium-K (1.46 %) were higher, with statistical differences compared to the 100 % fruit load (N: 1.77 %, P: 0.14 % and K: 1.02 %). In this study, it was evidenced that the concentration of sulfur-S was very sensitive to the increase in fruit load, where significantly differences were shown from the 30 % fruit load, and decreases of approximately 50 % in sulfur concentration were reported for trees with 100 % fruit load (0.10 % on average) compared to 20 % fruit load (S: 0.22 %). On the other hand, for the concentration of Mg, there were no statistical differences between the different fruit load intensities (Fig. 2).

This study analyzed whether different fruit load intensities influence the concentration of leaf micronutrients. For iron-Fe and zinc-Zn, there were no differences in relation to fruit load (Fig. 3a and d). For manganese-Mn, significant differences were detected, with 25 % reductions in the Mn concentration for 80 % fruit loads compared to lower fruit loads (Fig. 3b). Finally, for concentrations of copper-Cu the only significant difference was found for the high fruit load (100 %), with a 35 % decrease compared to the lower loads (20 and 30 %), with a mean concentration of 14.72 ppm (Fig. 3c).

Once it was identified that the high fruit load (100 %) affected the concentrations of essential nutrients, the content and stability of the total chlorophylls were performed to observe if there were alterations in this measurement (Fig. 4). Reductions were observed both for the content and stability- *CSI* of the total chlorophylls, with statistical differences for the 100 % fruit load compared with lower fruit load trees (20 and 30 %).

For the content of total soluble sugars in the leaves-*TSS*, it was shown that there is an inverse linear relationship between the content of *TSS* and fruit load, which indicates that greater fruit load in the coffee trees decreases the content of *TSS*. In this study, the lowest *TSS* content (mean of 8.37 mg g^{-1} DM) was reported for the 100 % fruit loads, while for the low fruit loads (20 and 30 %) the *TSS* content was 11.76 mg g^{-1} DM (Fig. 5). Likewise, we demonstrate the interrelationships between N, K, S and Cu with the leaf concentration of *TChl* and *TSS*, which were influenced by fruit load in this study (Fig. S4).

3.3. Effect of fruit load on the coffee yield components

It was observed that the number of fruits per branch increased with more significant fruit load, and differences were detected with 40 % fruit load compared to lower fruit load (20 %). However, the greatest number of fruits per branch was observed for the high fruit load (100 %), with an average value of 119 fruits per branch. A similar trend was reported for

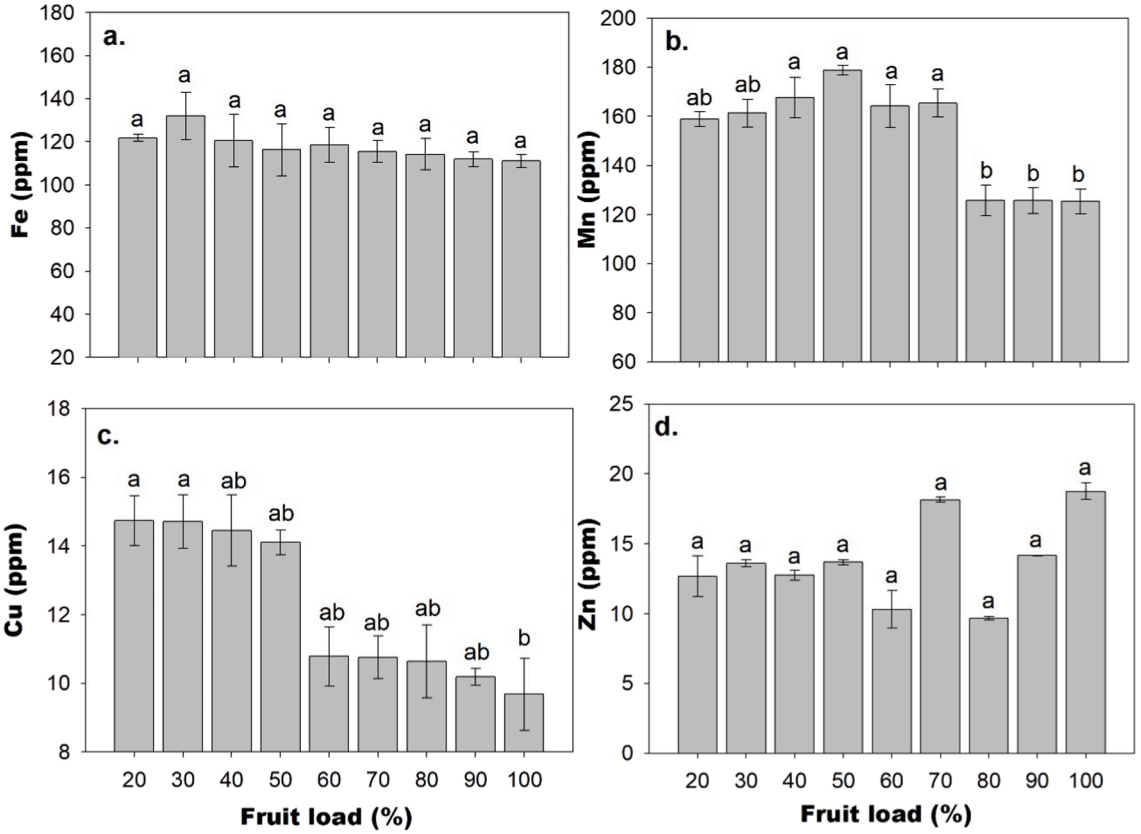

Fig. 3. Effect of the fruit load on leaf concentration of iron-Fe (a), manganese-Mn (b), cupper-Cu (c), and zinc-Zn (d). These measurements were made in fully developed leaves. Each data is the mean ($n = 3$) ± SE. The different letters denote significant differences among means within treatments according to the Tukey test ($p < 0.05$).

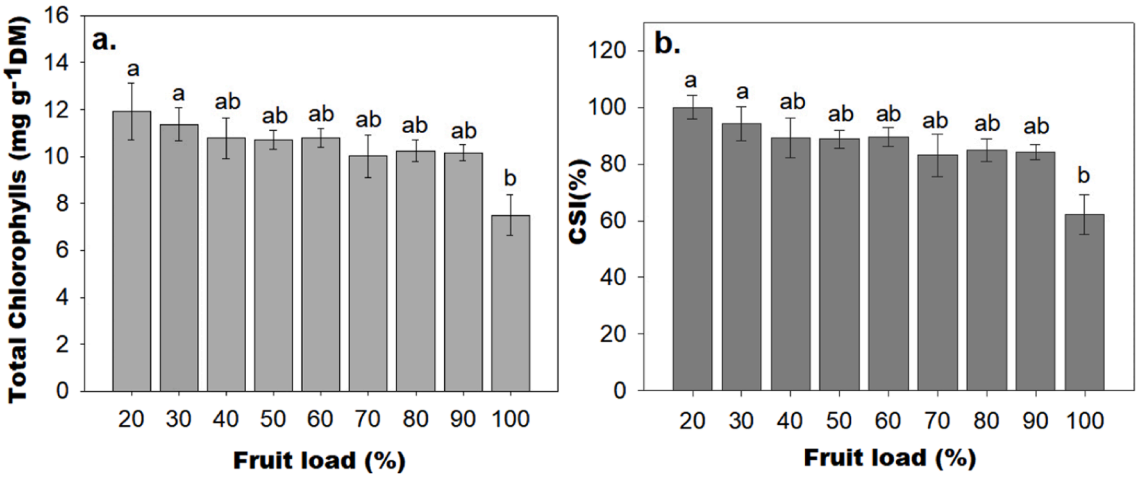

Fig. 4. Content of the total chlorophylls (a) and chlorophylls stability index-TCSI according to fruit load. These measurements were made in fully developed leaves. Each data is the mean ($n = 4$) ± SE. The different letters denote significant differences among means within treatments according to the Tukey test ($p < 0.05$).

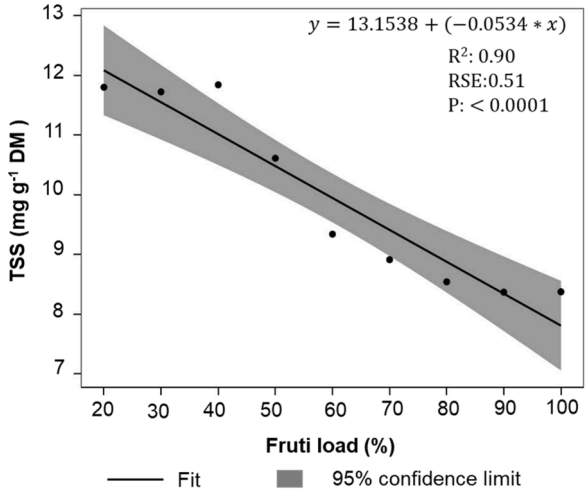

Fig. 5. Fit of linear model between the concentrations of total soluble sugars-TSS and fruit load measured in fully developed leaves. Values are reported as means ($n = 4$). RSE corresponds to residual standard error.

annual coffee production, whereby the 100 % fruit load had the highest mean annual production values, with 4.01 kg per plant, with significant differences compared to fruit loads below 60 % (2.4 kg plant^{-1}). However, the lowest production (1.9 kg plant^{-1}) was recorded for the treatment with 20 % fruit loads (Table 1).

On the other hand, the weight and diameter of individual coffee berries showed no statistical changes depending on the different fruit loads evaluated. In this study, coffee berries' average values of 2.18 g for weight and 14.80 mm for diameter were recorded throughout the experiment. In contrast, differences were found for the percentage of malformed fruits since the fruit load increase on the trees caused a deterioration in this respect. A high fruit load (100 %) showed statistical differences and an 8.5 % increase in the percentage of malformed fruits, compared to fruit load below 80 % (Table 1).

3.4. High fruit load modified the bean concentration of soluble sugars, organic acids, and alkaloids

For the interpretation of Fig. 6, low contents close to −1 are shown in blue, values relative to 0 indicate an intermediate range and are shown in green, and high values close to 1 are shown in yellow . With fruit loads from 50 to 80 %, intermediate to high values were obtained for caffeine,

Table 1
Effect of the fruit load on the fruit numbers per branch, production per plant, berry weight and diameter, and malformed fruits. Each data is the mean ± standard error-SE. The different letters denote significant differences among means within treatments according to the Tukey test ($p < 0.05$).

Fruit load (%)	Parameters				
	Fruits per branch	Production (kg plant^{-1})	Berry weight (g)	Berry diameter (mm)	Malformed fruits (%)
20	37.5 ± 2.46 c	1.92 ± 0.41 b	2.21 ± 0.03 a	14.78 ± 0.10 a	2.83 ± 0.75 b
30	48.75 ± 1.93 bc	2.17 ± 0.23 b	2.12 ± 0.03 a	14.74 ± 0.13 a	3.00 ± 0.73 b
40	58.75 ± 6.60 b	2.83 ± 0.32 b	2.20 ± 0.03 a	14.73 ± 0.11 a	3.17 ± 0.31 b
50	62.75 ± 1.65 b	2.54 ± 0.33 b	2.18 ± 0.04 a	15.03 ± 0.10 a	3.17 ± 0.87 b
60	87.75 ± 2.09 a	2.61 ± 0.16 b	2.14 ± 0.03 a	14.53 ± 0.11 a	3.17 ± 0.48 b
70	96.25 ± 2.87 a	3.08 ± 0.26 ab	2.24 ± 0.03 a	14.99 ± 0.09 a	3.17 ± 0.50 b
80	98.50 ± 2.78 a	2.73 ± 0.24 ab	2.17 ± 0.03 a	14.76 ± 0.19 a	3.33 ± 0.72 b
90	106.50 ± 5.60 a	2.79 ± 0.15 ab	2.20 ± 0.02 a	14.96 ± 0.13 a	5.33 ± 0.84 ab
100	119.50 ± 2.88 a	4.03 ± 0.29 a	2.16 ± 0.04 a	14.66 ± 0.13 a	8.50 ± 0.76 a

trigonelline, acetic acid, malic acid and quinic acid content. At high fruit loads (90 and 100 %), lower values were obtained for almost all biochemical compounds evaluated except for quinic acid.

With low fruit load (20–40 %), only intermediate to high values were reported for the concentration of soluble sugar (sucrose, glucose, and fructose), but low content was observed for acetic acid, quinic acid, malic acid and caffeine (Fig. 6). Thus, it was determined that the fruit load can influence the concentration of the bean biochemical compounds. There is an inverse linear relationship between the contents of soluble sugars and fruit load, which indicates that greater fruit load in the coffee trees decreases the content of total soluble sugars, with ranges from 95.80 to 79.90 g kg^{-1} DM for sucrose, 0.68 to 0.27 g kg^{-1} DM for glucose, and 0.39 until 0.21 g kg^{-1} DM for fructose (Fig. 7). However, it should be highlighted that the lowest values were determined for 100 % fruit load.

A quadratic type regression with respect to the fruit load was fitted for the contents of organic acids (citric, malic, acetic, lactic, and quinic)

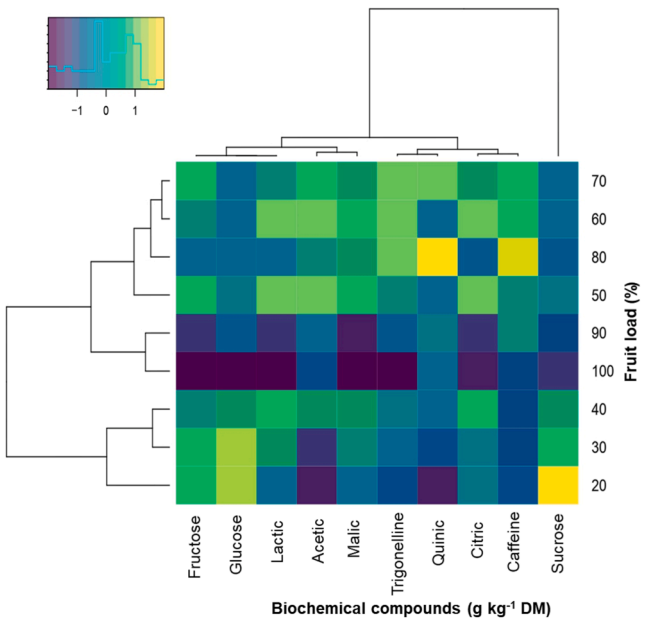

Fig. 6. Heatmap of the variability of the biochemical compounds determined in the coffee bean in relation to fruit load. For the interpretation, low contents close to −1 are shown in blue, values relative to 0 indicate an intermediate-range and are shown in green, and high values close to 1 are shown in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

evaluated in this study (Fig. 8). This indicates that the concentration of citric acid (10.39 g kg^{-1} DM on average), malic acid (5.57 g kg^{-1} DM), acetic acid (3.22 g kg^{-1} DM) and lactic acid (0.50 g kg^{-1} DM) were increased with fruit loads below 60 %. The greatest concentrations of quinic acid (8.16 g kg^{-1} DM) were reported at 80 % fruit load (Fig. 8). Nevertheless, the lowest values for all organic acids were found for the 100 % fruit load. Finally, the accumulation of bean organic acids evaluated in this study have the following order: citric > quinic > malic > acetic > lactic.

The high fruit loads (90 and 100 %) modified the concentration of bean alkaloids. A quadratic regression was adjusted for the contents of caffeine and trigonelline as a function of fruit load (Fig. 9). Increases were observed in the concentration of caffeine (mean of 11.708 g kg^{-1} DM) and trigonelline (7.34 g kg^{-1} DM) up to 70 % fruit load. However, 5 % decreases were reported for caffeine (10.60 g kg^{-1} DM) and 10 % in trigonelline (6.66 g kg^{-1} DM) for coffee trees with 100 % fruit load.

4. Discussion

In this papers, we address the effect of the different fruit load intensities on the photosynthetic performance, nutrient concentration, percentage of malformed fruits, and bean biochemical composition for the coffee "Cenicafé 1″ variety tree. These results can be a reference for predicting the effect of the fruit load on coffee trees, particularly when it has been identified that, at a certain plantation age, there is a high production concentration (Rendón, 2020; Unigarro et al., 2022). Furthermore, the coffee zone in Colombia uses the "Cenicafé 1″ variety, which is recognized for its high production potential and genetic resistance to coffee rust (*Hemileia vastatrix*) and *Colletotrichum kahawae*

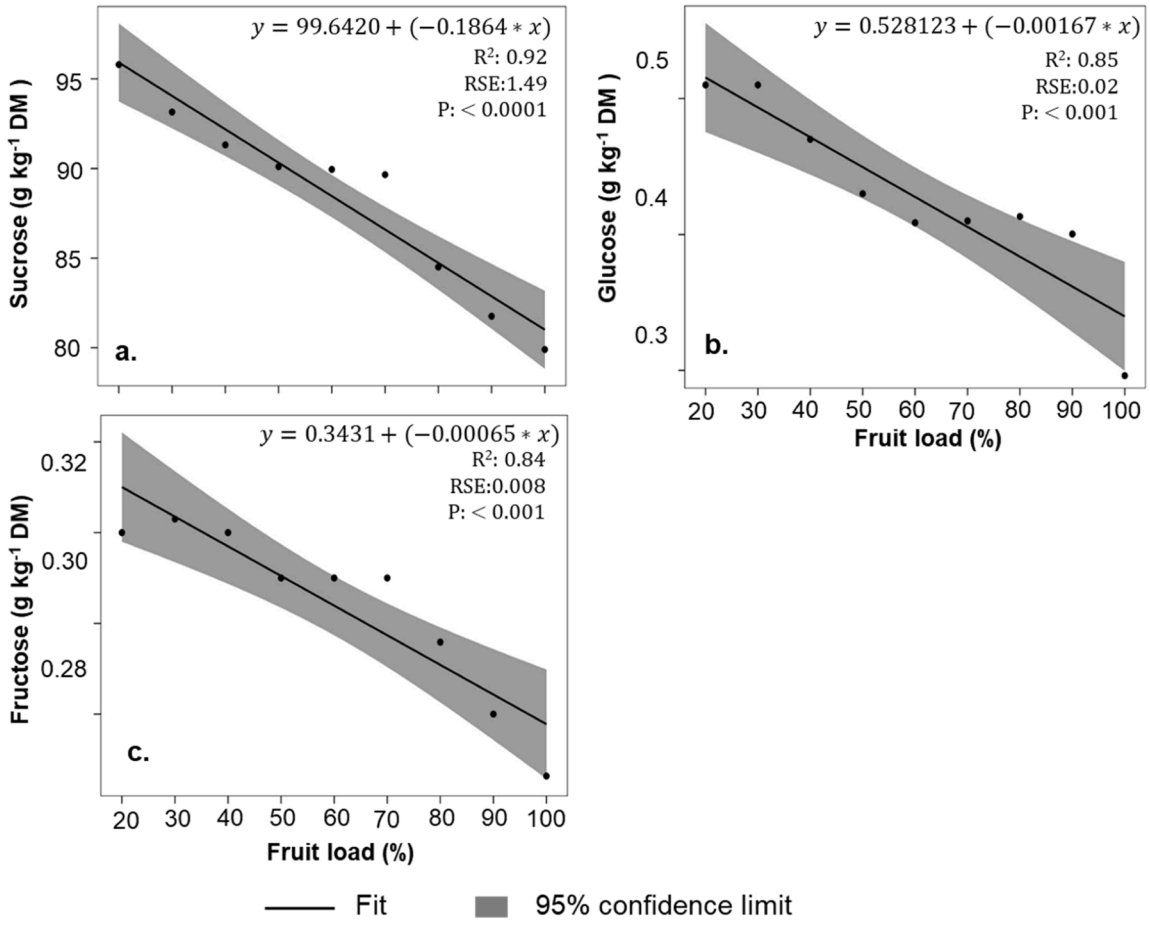

Fig. 7. Changes by fruit load on the accumulation of sucrose (a), glucose (b), and fructose (c). These compounds were measured in the coffee bean obtained from ripening fruits-BBCH88. Values are reported as means (n = 6). RSE corresponds to residual standard error.

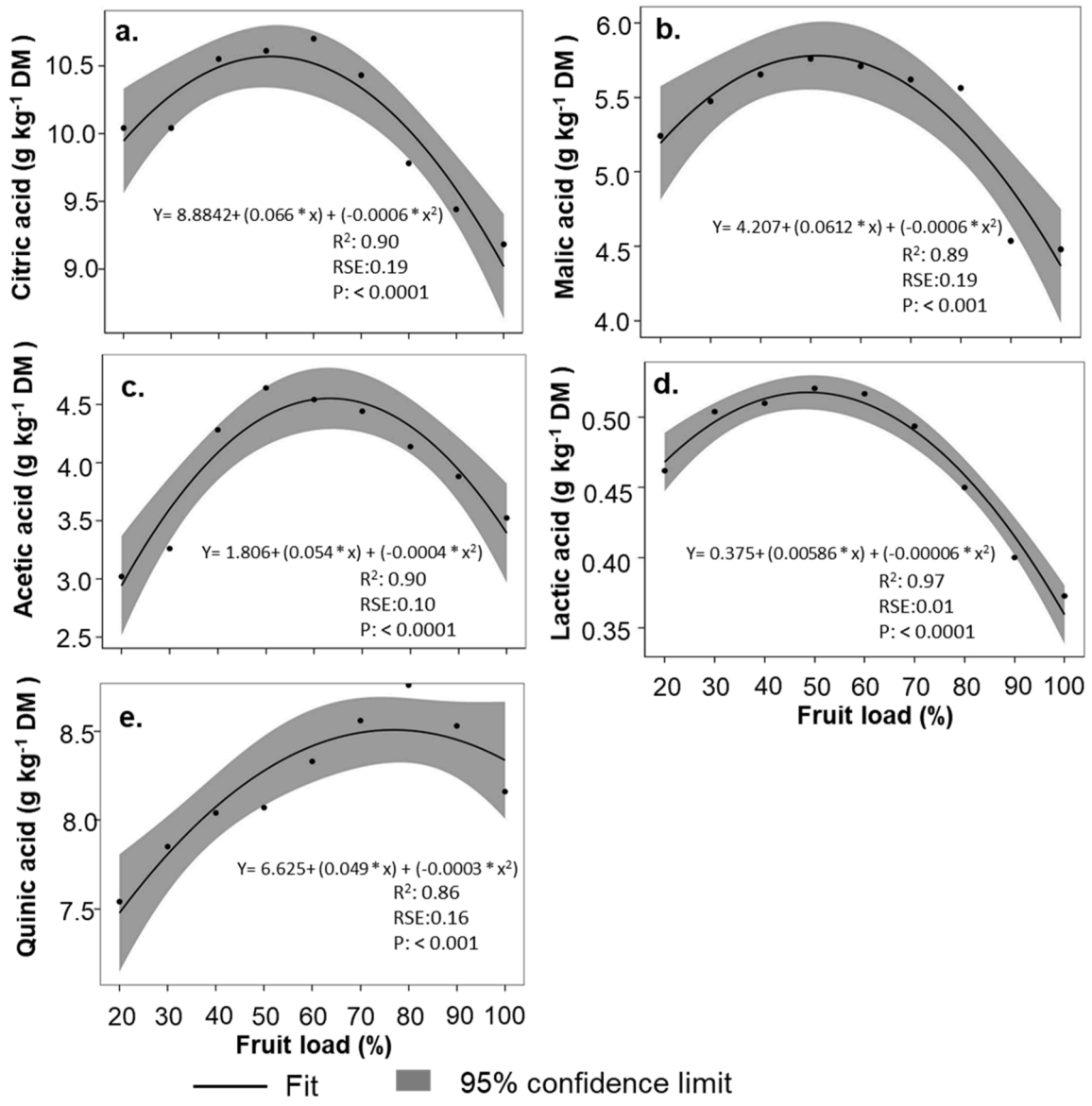

Fig. 8. Modifications by fruit load on the bean organic acids content. The concentrations of Citric acid (a), Malic acid (b), Quinic acid (c), acetic acid (d), and Lactic acid (e) were determined. These compounds were measured in the coffee bean obtained from ripening fruits-BBCH88. Values are reported as means ($n = 6$). RSE corresponds to residual standard error.

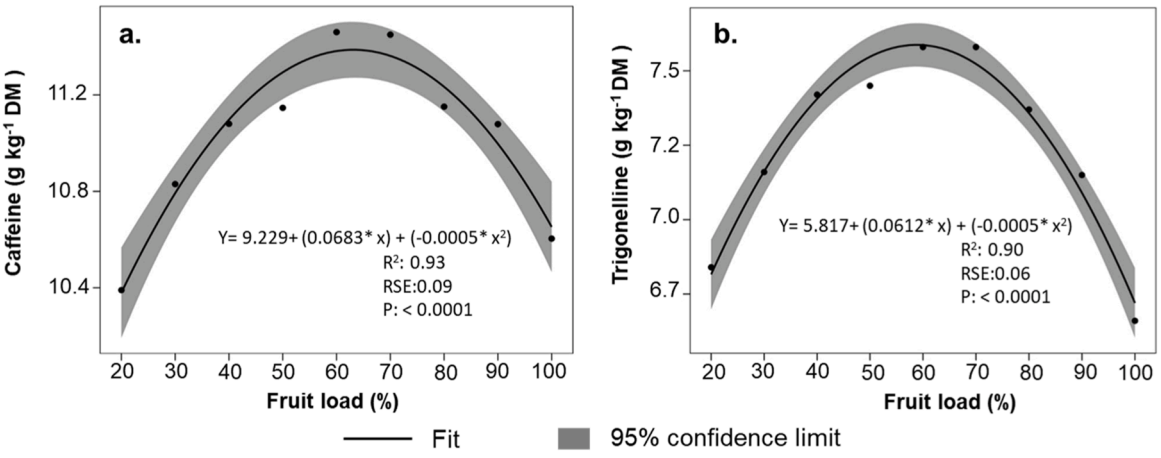

Fig. 9. Concentrations of caffeine (a) and trigonelline (b) in relation to fruit load. These compounds were measured in the coffee bean obtained from ripening fruits-BBCH88. Values are reported as means ($n = 6$). RSE corresponds to residual standard error.

(Maldonado and Giraldo, 2020), but which may be vulnerable to the effects of high fruit load. This information constitutes a tool to seek or promote strategies for crop management that can reduce the impact of high fruit load in field-grown coffee crops that are established in full sunlight. This problem has been frequently reported for equatorial or tropical regions (DaMatta et al., 2007).

In the leaf gas exchange measurements, it is evident that there are no effects on the part of the fruit load, while the phenological stages of fruit development showed significant alterations as a result of fruit load (Fig. 1). This same result was reported by DaMatta et al. (2008) and Avila et al. (2020), where the different fruit loads evaluated did not demonstrate statistical changes in the gas exchange parameters *A*, g_s, and C_i. In this way, Ávila et al. (2020) and Unigarro et al. (2022) demonstrate a change in photosynthetic rates depending on the different stages of fruit development. The lower values of *A* and g_s reported for BBCH71 are associated with slow fruit growth and, during this stage, low quantities of carbohydrates for the fruit pericarp cell division are required (De Castro and Marraccini, 2006; DaMatta et al., 2007). In addition, the coffee berry has photosynthetic activity and may be able to guarantee the carbon requirements during this growth phase (Vaast et al., 2005; Ocampo et al., 2010).

On another note, the VPD has been linked to low photosynthetic rates, as has been previously reported in coffee plants, where it was identified that there are 34 % decreases in photosynthetic rates with values of VPD of 2.4 KPa (Almeida et al., 2021). In this way, we confirmed that low *A* at the phenological stage of fruit development-BBCH71 may relate to the VPD, which registered values of 2.6 KPa (Fig. S1). Meanwhile, for the phenological stages -BBCH73 and BBCH79 (linear fruit growth or filling phase), the increase in *A* corresponds to a stimulus by the strongest sink strength of the fruits to guarantee the carbon requirements for import and utilization of photoassimilates, and then allocation to the cellular elongation processes and metabolic activity for the endosperm development (De Castro and Marraccini, 2006; Ocampo et al., 2010). Furthermore, we also evidenced in this study that increases in photosynthetic rates were related to higher g_s, which may be linked to fruit load increase (Fig. 1b), as has been previously reported in coffee *C. arabica* plants (DaMatta et al., 2008; Unigarro et al. al., 2022).

In the BBCH88 stage, the parameters C_i, *E*, and WUE_e showed significant decreases (Fig. 1c, d, and e), since in this period the carbohydrate storage phase begins for beans, which has been characterized by reductions in respiratory rates and dry mass accumulation (Ságio et al., 2013; Osorio Pérez et al., 2023a). Therefore, the sink strength of the coffee fruits may be regulated by the start of the biochemical changes that generate modifications in the color of the pericarp and imports of sugars and organic acids into the beans (De Castro and Marraccini, 2006; Ságio et al., 2013). However, it was evidenced that decreases are more marked for fruit load of 100 %, which reflects the fact that these plants can trigger stress conditions for reasons other than stomatal limitations (Farquhar and Sharkey, 1982; Pillitteri and Torii, 2012). These may be related by the source-sink conditions, which were estimated at around 6.47 cm^2 leaf area per fruit for fruit load of 100 % compared to the lower fruit load (20, 30, 40 and 50 %), with mean values of 20.81 cm^2 leaf area per fruit during the BBCH79 stage (data not shown).

The elevated fruit load evaluated in this study (100 %) shows changes in macronutrient concentrations compared to other fruit loads. The significant decreases in N, P, K, and S evidenced the high demand by fruits for these nutrients during the filling phase, as has been previously reported by Laviola et al. (2009) and Sadeghian et al. (2012). However, we confirmed that, for N (1.77 %), K (1.02 %) and S (0.10 %), the concentrations are very low in relation to the nutritional sufficiency level reported for *C. arabica* (N: 2.3–2.8 %, K: 1.5 to 2.0 % and S: 0.15–0.19 %). Therefore, we considered nutritional deficiencies or limitations with 100 % fruit load (Sousa et al., 2018; Sadeghian, 2020). The nutritional deficiencies of K and S for elevated fruit load (100 %) constitute interesting data that confirms the strong sink strength of coffee berries and supplements results previously reported, which only evidenced nitrogen nutritional deficiencies (Bote y Vos, 2016; Avila et al., 2020).

Nitrogen deficiencies (Fig. 2a) have been previously reported in coffee trees with a high fruit load (Chaves et al., 2012; Bote and Vos, 2016; Avila et al., 2020) and are related indirectly to the remobilization of nitrogen compounds from source to sinks by the fruits, and this can be decreased by the photosynthetic pigments biosynthesis from source leaves (de Bang et al., 2020). Our results evidenced alterations in the concentration and stability-*CSI* of total chlorophylls (Fig. 4) and their interrelationships with the leaf content of N ($r = 0.50$) and K $r = 0.72$ (Fig. S4), which were influenced by different fruit load intensities. The above indicates indirectly that more oxidative stress at the cellular level, low photosynthetic efficiency, and affection of carbon metabolism during the endosperm-filling stage may be triggered (Pompelli et al., 2010; Souza et al., 2020). These results have been associated to coffee trees since, in the high fruit load branches, a low autonomy for allocating carbohydrates and nutrients in the periods of greatest demand by the fruits, generally during the endosperm filling, was demonstrated (Chaves et al., 2012).

As expected, potassium is the nutrient that coffee fruits accumulate in the greatest quantities (Sadeghian, 2022) and significant decreases were confirmed for 100 % fruit load, with nutritional deficiencies at leaf level (Fig. 2c). Potassium has a pivotal role in the activity of source leaves photosynthetic production and limitations in this nutrient can affect the production of photoassimilates (de Bang et al., 2021). In this regard, we evidenced that the leaf concentration of total soluble sugars-TSS was affected (Fig. 5), with a positive correlation ($r = 0.62$) with leaf concentration of K (Fig.S4).These results can be associated with one another, given that potassium is related to enzymes of the sugar metabolism or ATPase that are linked to active metabolic processes at the cellular level and the opening of the stomata (Wind et al., 2010; Tognetti et al., 2013; Taiz et al., 2015).

Leaf concentrations of Mn and Cu were found to decrease with fruit loads greater than 80 % (Fig. 3). Sadeghian (2022) and Laviola et al. (2009) showed that the accumulation of these micronutrients has a relationship with the coffee fruit development and concluded that the highest concentrations coincide with the endosperm-filling stage. However, our results confirmed only nutritional deficiencies for the leaf concentration Cu (9.67 ppm on average) compared with reference values for *C.arabica* of 14–20 ppm (Martinez et al., 2003). With the reductions of both Mn and Cu resulting from fruit load increase, modifications in the photosynthetic efficiency at the photosystems level were expected, since these nutrients are part of the structure of the superoxide dismutase-SOD, which has functions in ROS detoxifying at the cellular level and regulation of redox homeostasis (Hänsch and Mendel, 2009; Andresen et al., 2018). Likewise, ours results confirmed the role of Cu in the leaf concentration of *TChl* and *TSS* (Fig. S4).

The increase in fruit load resulted in higher production per plant, and this result has been previously reported for *C. arabica* trees with high fruit load (DaMatta et al., 2008; Unigarro et al., 2022). Bote and Vos (2016) determined that yield increased linearly with an elevated fruit load on the coffee trees. These results have been associated with the source strength and sink by the fruits that supports the variations in total crop load (DaMatta et al., 2008). Since the berry photosynthetic area represents up to 20 % of the total photosynthetic area for a tree with elevated fruit load, this can contribute to their daily respiration cost and carbon requirements (Vaast et al., 2005) and thus ensure higher production, as has been reported for 100 % fruit load (Table 1).

Although preliminary, our results show that fruit size measured indirectly through the weight and equatorial diameter of the individual berries is not altered by the different intensities of fruit load. We concluded that the strong sink strength of the coffee fruit of variety "Cenicafé 1″ trees is more associated with the number of fruits per branch than the fruit size (Table 1). Vaast et al. (2005) reported no variations in the fruit weight of five-year-old *C. arabica* "Costa Rica 95″

variety plants. In this regard, changes to the coffee fruit size are more associated with the degree of shade, as previously described by Vaast et al. (2006). Finally, the bean filling was affected by the fruit load increase, evidenced by the percentage of malformed fruit, with values from 2.8 to 8.5 %. However, the most critical and significant increases were demonstrated for a high fruit load of 100 % (Table 1). This same result is reported by DaMatta et al. (2008) in ten-year-old coffee "Catuaí" variety trees. This is associated with the high carbohydrate translocation promoted by elevated fruit load branches (Chaves et al., 2012).

The influence of high fruit loads on the bean biochemical composition, as observed for fruit loads of 90 and 100 % (Fig. 6), has been previously reported for *C. arabica* (Bertrand et al., 2006; Vaast et al., 2006). We confirmed that there is a linear relationship between the content of sucrose, glucose, and fructose with the increase of fruit load (Fig.7). These low levels of soluble sugars for beans can be associated with the decrease of leaf TSS as a function of fruit load (Fig. 5), these being compounds related to the primary product of leaf photosynthesis, which are then phloem loaded and transported to the sink organs (Bihmidine et al., 2013; Taiz et al., 2015). Moreover, the reductions in the leaf concentrations of potassium reported in this study (Fig. 3) may be linked to the low content of soluble sugars, findings that have also been reported by Clemente et al. (2015) and Vinecky et al. (2016) with low doses of potassium fertilizers for coffee bean.

The concentrations of glucose and fructose reported in this study correspond to a stimulus by the low sucrose content, which has been reported as a signaling molecule at the cellular level that triggers the hydrolyzing of monosaccharides (glucose and fructose), particularly in sink organs (fruits) for the maintenance of respiration costs (Tognetti et al., 2013; Taiz et al., 2015). Furthermore, the low concentrations of glucose and fructose may be linked to a decrease in the contents of organic acids for beans, as has been reported with fruit loads greater than 80 % (Fig. 8). These two findings may be associated, since monosaccharides are metabolic substrates for the biosynthesis of organic acids for beans (Araújo et al., 2012). These compounds within coffee beans have been reported in the following metabolic pathways: 3-deoxy-d-arabino-heptulosonate-7-phosphate, fructose-1.6-bisphosphate, fructose-6-phosphate, glucose-6-phosphate, phosphoenolpyruvic acid and UDP-glucose (Koshiro et al., 2006; Koshiro et al., 2015).

The decrease in caffeine and trigonelline may be linked to nitrogen and potassium deficiencies reported in this study (Fig. 3), findings also reported by Vinecky et al. (2016) and Ahmed et al. (2021) with low doses of nitrogen and potassium fertilizers. Likewise, Vaast et al. (2006) reported reductions in caffeine and trigonelline with elevated fruit load for coffee "Costa Rica 95″ variety trees. The reductions of sugars, organic acids, caffeine, and trigonelline in coffee beans evaluated in this study, are the first reported effect of elevated fruit load on the bean biochemical composition in field-grown trees and full sun established trees (Läderach et al.,2010; Bote and Vos, 2017). These results indicate that elevated fruit load may have negative effects on coffee beverage quality, since these compounds have been correlated with sensorial attributes (Koutouleas et al., 2022; Osorio Pérez et al., 2023a; 2023b). However, more research is still needed to corroborate this effect, especially under field-grown trees conditions and full sun establishment.

5. Conclusions

The results of our study suggest that high fruit loads on C. *arabica* "Cenicafé 1″ variety trees affected the leaf nutrient concentration, chlorophylls, and soluble sugars, as well as the bean biochemical composition. We have provided evidence to support the hypothesis that varying the fruit load did not influence leaf photosynthetic activity but rather the phenological stage of fruit development-BBCH. Also, nutritional deficiencies of nitrogen, potassium, sulphur and copper during the endosperm-filling stage were determined by low autonomy in high fruit load branches. Thus, manipulating the sink organs through varying fruit load intensities makes it apparent that fruits are dominant sinks with a high capacity for aggravated leaf nutritional limitations in source leaves. In this way, we demonstrate the interrelationships between nutritional deficiencies and the leaf concentration of *TChl* and *TSS*, which were decreased in the 100 % fruit load. Finally, the increase in the fruit load modified the biochemical composition of the coffee beans. It was found that sucrose, glucose, and fructose linearly decreased with fruit load in beans. In addition, with elevated fruit load, the accumulation of organic acids, caffeine, and trigonelline was altered. Hence, we confirm that coffee fruits are highly competitive sink organs with strong imports of carbohydrates and nutrients, which eventually can provide a negative imbalance in the supply of photosynthetic products in source leaves.

CRediT authorship contribution statement

Andrés Felipe León-Burgos: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **José Raúl Rendón Sáenz:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Luis Carlos Imbachi Quinchua:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Carlos Andrés Unigarro:** Writing – original draft, Methodology, Investigation, Conceptualization. **Valentina Osorio:** Methodology, Conceptualization. **Siavosh Sadeghian Khalajabadi:** Conceptualization, Writing – original draft. **Helber Enrique Balaguera-López:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113005.

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