PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2025.028 PHOTOSYNTHETICA 63 (3): 297-308, 2025

Evaluating physiological traits of *Coffea canephora* clones intercropped with *Hevea brasiliensis*

I.D.P. PEREIRA*, S. DOUSSEAU-ARANTES**, J.A. MACHADO FILHO**, L.O. ARANTES**,
M.M. MENDES*, V.F. SANTOS*, G.A.R. DE SOUZA**, B. CERRI NETO**, D. CASSOL***,
and A.R. FALQUETO*,+

*Department of Agrarian and Biological Science, Federal University of Espírito Santo, 29932-540 São Mateus, ES, Brazil**
*Capixaba Institute of Research, Technical Assistance and Rural Extension, BR 101N, Km 15, Brazil***
*Department of Botany and Plant Sciences, University of California at Riverside, 1207E Riverside, CA, USA****

Abstract

In this study, we evaluated the physiological response of the photosynthetic apparatus [using chlorophyll *a* fluorescence (Chl*a*F) measurements], changes in leaf nutrient contents, and productivity of 16 *Coffea canephora* clones grown alone (NC, full sunlight) or intercropped with *Hevea brasiliensis* (IC, shaded). Shade from *H. brasiliensis* trees influenced the physiological performance of coffee plants. Some of these coffee clones achieved clear responses to shading by rubber trees, indicating that the responses of coffee plants to intercropping are genotype-specific. The PSII complex of the NC plants was more susceptible to photoinhibition, especially clones 02, 73, 143, and 109A, which had increased minimal fluorescence, specific energy fluxes per reaction centers, maximum photochemical quantum yield, quantum efficiency of electron transfer from Q_A^- to the electron transport chain beyond Q_A^-, and number of active PSII reaction centers per cross section, performance index for conservation of energy from captured excitons to reduction of intersystem electron acceptors, and lower maximum fluorescence. In contrast, the higher photosynthetic efficiency and productivity of the clones under shaded conditions indicated their potential for cultivation together with *H. brasiliensis*.

Keywords: JIP-test; leaf nutrients; photosystem II; shade.

Introduction

Coffee is cultivated in more than 80 countries and is one of the most important agricultural sectors (Semedo *et al.* 2018). In 2020 and 2021, South America produced approximately 77.5 million coffee bags, ranking one of the four leading coffee-producing regions globally (ICO 2022). In 2021, Brazil produced 16.29 million coffee bags,

Highlights

- ● Light intensity differentially modulates the physiology of coffee clones
- ● In full sunlight, photoinhibitory damage occurs to the PSII complex
- ● Intercropping has positive effects on PSII photochemistry

Received 14 March 2025
Accepted 17 September 2025

+Corresponding author
e-mail: antelmofalqueto@gmail.com

Abbreviations: ABS/RC – energy absorption flux per reaction center; Chl*a*F – chlorophyll *a* fluorescence; CS – cross section; DI_0/RC – energy flux dissipated per reaction center; ET_0/RC – energy transport flow per reaction center; F_0 – minimal fluorescence yield in the dark-adapted state; F_m – maximal fluorescence yield in the dark-adapted state; $PI_{(abs)}$ – performance index for conservation of energy from captured excitons to reduction of intersystem electron acceptors; RC – reaction center; RC/CS_0 – number of active PSII reaction centers per cross section; TR_0/RC – energy flow captured by reaction center; φE_0 – quantum efficiency of electron transfer from Q_A^- to the electron transport chain beyond Q_A^-; φP_0 – maximum photochemical quantum yield.

Acknowledgments: We thank the Capixaba Institute for Research, Technical Assistance and Rural Extension (INCAPER), through project number 02.13.02.013.00.00, financed by the Brazilian Agricultural Research Corporation (EMBRAPA), for the use of the Sooretama Experimental Farm (FES). We also thank the Espírito Santo State Research and Innovation Support Fund (FAPES), the Office to Coordinate Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq), which in partnership with Federal University of Espírito Santo (UFES), granted resources to the Laboratory for Plant Ecophysiology (LEV), where the experiment was carried out.

Conflict of interest: The authors declare that they have no conflict of interest.

setting a new record, 13.8% greater than in 2020 (CONAB 2021). Coffee consumption in the country grew by 1.71% in 2021, mainly consumption of national coffee types (ABIC 2021). In Espírito Santo State, coffee is grown in about 80% of the municipalities, with about 273.7 thousand hectares cultivated (CONAB 2021).

Plants are exposed to different seasonal light intensities during the year. The high intensities associated with high air temperature during the summer reduce the crop performance, because the evapotranspiration rates and the water vapor deficits increase (Medauar *et al.* 2021). As a consequence, lower efficiency of the electron transport chain (ETC) lead to increases in oxidative pressure on chloroplasts due to overproduction of reactive oxygen species (ROS), specifically in the electron acceptor side of PSI associated with the thylakoid membrane (Gill and Tuteja 2010), which has been reported to reduce the vegetative and reproductive development (DaMatta *et al.* 2016).

The state of Espírito Santo has regions with low and irregular rainfall distribution, particularly including water deficit lasting approximately five months, coinciding with the winter season (Lorençone *et al.* 2024). Most climate change scenarios envisage physiological impairment of coffee trees, which can affect carbon assimilation processes, mainly through stomatal closure, which is frequent under abiotic conditions (Rodrigues *et al.* 2016). Hence, there is a need for research on strategies aimed at understanding the physiological processes of coffee crops under potentially more severe conditions.

Intercropped conilon coffee cultivation has become an advantageous alternative, recommended to mitigate damage to coffee trees caused by adverse weather (Gomes *et al.* 2020). This is particularly useful for places with large variations in climatic conditions, which generate a huge impact on coffee quality and yield (Peloso *et al.* 2017), especially in the critical grain-filling stage (October to March) (Partelli *et al.* 2013). To mitigate possible damage, producers need to alter the management of their crops in order to attenuate abiotic effects that affect the plants. In this respect, intercropping can provide extra income for producers through the possibility of using species such as rubber trees (*Hevea brasiliensis*), which have little competition with the coffee trees due to their adaptive characteristics, rusticity, upright crown, and deep root system (Nunes *et al.* 2021).

The combination of *Coffea canephora* with *Hevea brasiliensis* influences the microclimate and development of coffee trees, increasing relative humidity and attenuating the temperature and irradiance, characteristics of the hotter season of the year (Araújo *et al.* 2016). A recent study reported increased production of four varieties of conilon coffee grown under low irradiance (Assis *et al.* 2019). Biochemical and physiological changes in two conilon genotypes were studied, and the authors found genetic divergence between the coffee genotypes under artificial shade levels (Ferreira *et al.* 2021). These authors also reported a decrease in the contents of carbohydrates, amino acids, and phenols with a greater reduction in light intensity. Finally, shading can positively impact the growth, productivity, ecological, and microclimatic variables of conilon coffee plants, as verified by Piato *et al.* (2020), who also reported that physiological and photosynthetic traits were positively affected by shade.

Despite previous research, it is still necessary to carry out ecophysiological studies of clonal varieties in order to verify their behavior when submitted to intercropping in agroforestry systems *vs*. full sunlight in monocropping, to support management strategies. Therefore, this study evaluated the physiological responses of 16 clones of *Coffea canephora* under two conditions: intercropping with *Hevea brasiliensis* (IC) and no intercropping (NC), by comparing the results obtained involving Chl*a*F (JIP-test), foliar nutrient variation, and yield. Furthermore, we report under which conditions the clones had better photosynthetic performance (higher productivity), and indicate trends for the clones to benefit or not in relation to the IC and NC treatments.

Materials and methods

Study site, plant material, and climate: The study was conducted at the Sooretama Experimental Farm of the Capixaba Institute of Research, Technical Assistance, and Rural Extension (Incaper, Linhares, Espírito Santo State, Brazil). The local climate is classified as Aw – tropical with dry season (Köppen-Geiger), with an average annual temperature of 23.8°C and average yearly precipitation of 1,200 mm. The soil is classified as a cohesive, dystrophic, red-yellow latosol. The 16 conilon coffee clones were submitted either to a non-intercropped treatment (NC) or intercropped with *Hevea brasiliensis* (IC), in equal areas of 1,100 m². The clones evaluated in the study were designated 02, 03, 14, 16, 19, 31SE, GG, 73, 83, 99, 120, 143, 153, 104A, 109A, and S143, and were randomly distributed in four plots of both treatments. In each plot, four individuals were evaluated, excluding those at the edges. Adult individuals of *H. brasiliensis* with a height of 15 to 20 m were used. In the NC treatment, the trees were arranged laterally to the intercropped plants at a distance of approximately 10 m. The spacing of *H. brasiliensis* was arranged in 4.0 × 2.5 × 30 m, and the spacing of coffee plants was 2.5 × 1.0 m. The climatic conditions during the study period were monitored through the local meteorological station.

ChlaF (JIP-test): Sampling was performed between September 2018 and September 2019 in the four seasons. Chl*a*F measurements were performed using a portable fluorometer *Handy-PEA* (*Hansatech Instruments Ltd*., King's Lynn, UK) during the morning (from 7:00 to 10:00 h) on fully expanded young leaves (3rd or 4th pair of leaves from plagiotropic branches from the apex), previously dark-adapted for 30 min, sufficient time for complete oxidation of the photosynthetic electron transport system. Afterwards, a flash of light (650 nm) was emitted with a pulse of 3,000 μmol(photon) m^{-2} s^{-1} on the leaves for 1 s. Data acquisition with *Handy-PEA* was performed at the following intervals: 10 μs (from 10 to 300 μs), 0.1 ms (0.3 to 3 ms), 1 ms (3 to 30 ms), 10 ms (30 to 300 ms), and

100 ms (300 ms to 1 s). These fluorescence signals were used to calculate the parameters of the JIP-test (Strasser and Strasser 1995).

Leaf chemical analysis: The leaf collection for nutritional analysis was carried out in October 2019, involving the removal of 20 fully expanded leaves from random individuals of the 16 clones in each plot of both treatments (3rd or 4th pair of leaves from plagiotropic branches from the apex). After sampling, the leaves were dried in a forced-circulation oven at 70°C until a constant mass and then were crushed in a mill. The macro [g kg^{-1}] and micronutrient [mg kg^{-1}] concentrations (N, P, K, Ca, Mg, B, Zn, Mn, Fe, and Cu) of leaf samples were quantified using two solubilization methods, nitric perchloric acid for nitrogen and sulfuric acid for the other nutrients (Carmo *et al*. 2000).

Productivity: The total mass of the beans was measured through the sum of each coffee clone in both IC and NC treatments. Subsequently, samples of approximately 2 kg were taken for drying in the forced-circulation oven at 48°C. Next, it was performed to determine the yield [kg ha^{-1}], which was estimated through the ratio between the mass of cherry fruits in relation to dry beans with 13% moisture. The productivity and the crushed mass results were plotted as kg per plant and g, respectively.

Statistical analysis: The experimental design was completely randomized in a factorial scheme, composed of 16 coffee clones and two treatments (IC and NC). Chl*a*F (JIP-test), leaf chemical analysis, and productivity data were submitted to analysis of variance (*ANOVA*). The means were compared by the *Scott-Knott* test ($p<0.05$). For these comparisons, we used the *Sisvar* software *version 5.6*. Also, data from all seasons of the year were submitted to principal component analysis (PCA) using *R CRAN version 4.1.2* (R Core Team 2020).

Results

ChlaF (JIP-test): The first Chl*a*F sampling was carried out during the spring (10 September 2018), during which 210 mm of rain was recorded. This precipitation value was the highest recorded among all seasons. The maximum and minimum temperatures were 29 and 20°C, respectively (Fig. 1). At that time, the coffee trees were in the flowering stage. The second data collection was carried out in the summer (16 February 2019), when precipitation was 52.2 mm, with maximum and minimum temperatures of 35 and 22°C. The highest temperature was the maximum among all seasons (Fig. 1), and the plants were in the grain-filling stage. The third collection was performed in autumn (16 May 2019). Autumn rainfall was 68.6 mm, and maximum and minimum temperatures were 31 and 20°C (Fig. 1). At that time, the grains were already in the maturation stage. The last collection was in winter (19 September 2019), with the lowest monthly rainfall among all seasons, with maximum and minimum temperatures of 30 and 18°C (Fig. 1), the lowest temperature in all seasons. When the measurements were made, the harvest and pruning of the trees had already taken place.

In the multivariate analysis covering all seasons of the year for Chl*a*F (Fig. 2), 82.6% of the total variance of the data was observed, making it possible to form seasonal groups of spring, summer, and autumn. Winter was not grouped with the others, so we performed PCA for each season separately to ascertain the possible reasons for the formation of the observed clusters.

In spring, principal component analysis revealed 78.5% of the total variance of the data (Fig. 3*A*). The first principal component (PC1) was responsible for 63.5% of the variance for the 11 parameters and clones analyzed. There was a single group formed by the NC treatment, mainly for clones 02, 16, 99, 109A, and 31SE. The parameters that most contributed to the formation

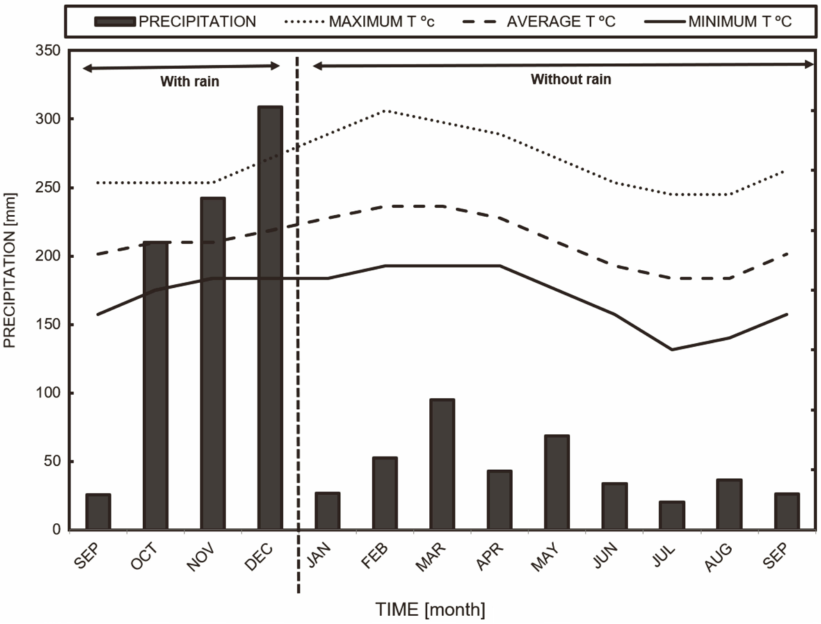

Fig. 1. Monthly climate conditions.

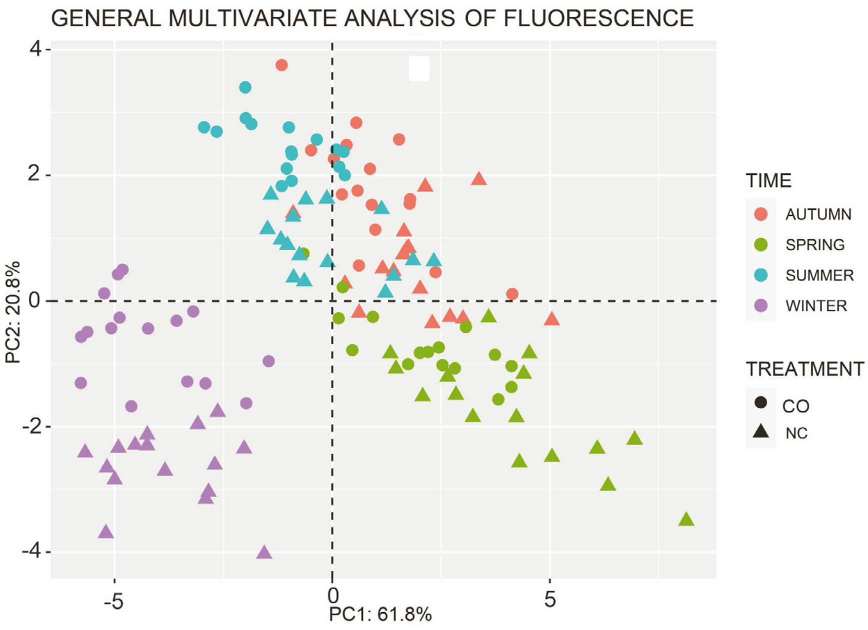

Fig. 2. Graphical dispersion of *Coffea canephora* showing the JIP-test parameters for the intercropped (IC) and non-intercropped (NC) treatments submitted to principal component analysis (PCA) at all times of the year. *n* = 20.

of this cluster were F_0, φD_0, TR_0/RC, and DI_0/RC. Clone 02 NC had the highest values of the Chl*a*F parameters evaluated, which differed significantly from those obtained for the IC treatment, followed by 99, 16, and 109A, which, for the same treatment, showed significant means only for the TR_0/RC and DI_0/RC parameters (Table 1S, *supplement*). The second principal component (PC2) explained 15% of the variability (Fig. 3*A*). The parameters that most contributed to the clustering of clones in the IC treatment were F_m, φP_0, φE_0, and mainly RC/CS_0. The clones that had the highest significant means in relation to the IC treatment were 16 and 83 (Table 2S, *supplement*). $PI_{(abs)}$ had one of the smallest vector dimensions and was positioned at the center of the overlap area in the graph, which is explained by the presence of very heterogeneous mean values (high variance).

In the summer, the two main components explained 74.3% of the total variation of the data. The first principal component explained 54.4% of the data variance (Fig. 3*B*). There was a cluster of 13 clones for the IC treatment. However, for the same treatment, clones 16, 02, 104A, and 83 were positioned in the extreme right-hand region of the graph, and the Chl*a*F parameters that best explained this formation were φD_0 and DI_0/RC. Clone 109A, although located in an intersection area, was the clone that most showed significant differences in ABS/RC, DI_0/RC, and φD_0, which are considered nonphotochemical parameters, followed by 104A, 02, 16, and 83 (Table 3S, *supplement*). In the lower central region, there was a clustering of clones GG, 14, 31SE, 03, S143, 120, 143, 73, and 19 (Fig. 3*B*). These clones did not have significant statistical differences (Tables 3S and 4S, *supplement*) between the IC and NC treatments, which probably resulted in the positioning of these clones without direct influence of specific variables. The second principal component (PC2) explained 19.9% of the variance of the data referring to all the Chl*a*F parameters and clones analyzed for the intercropped treatment, with clustering of clones 109A, 19, 73, 03, 104A, and 83 (Fig. 3*B*). The clustering of these clones can be explained by the high means with statistically significant differences (Table 4S) in relation to the parameters F_m, RC/CS_0, φP_0, φE_0, and $PI_{(abs)}$. Clone 104A had the highest values of some of these Chl*a*F parameters, followed by 03, 109A, and 73. Clone 31SE was positioned in the upper right region of the graph, which can be explained by the significant values of the parameters F_m and F_0 (Tables 3S and 4S).

Fig. 3*C* shows the PCA obtained for the autumn season, with total variance of 71.5%, which also made it possible to carry out selection of parameters and clones for the IC and NC treatments. Principal component 1 (PC1) explained 52% of the data variance, and clones 143, 104A, 99, 153, 73, 2, GG, 16, 19, 130, S143 formed a cluster in the NC treatment. Clones 143 and 104A had a strong influence from F_0, TR_0/RC, and ABS/RC and were positioned in the upper right region of the graph for this treatment. For NC, clones 99, 153, 73, 02, and GG were strongly correlated with DI_0/RC, φD_0, and ET_0/RC. Still for the NC treatment, clones 16, 19, 120, and S143 were located in the lower left region of the graph. What made this arrangement possible, in general, was the absence of significant differences of those parameters in 143, 104A, 31SE, and 03, which were respectively the clones with the highest average NC values in relation to autumn (Table 5S, *supplement*). Principal component 2 (PC2) was responsible for 19.5% of the data variation (Fig. 3*C*). There was a cluster formed by clones 19, 03, 31SE, 120, 16, 73, and S143 in the intercropping area, where the parameters that most contributed to the formation of the cluster were F_m, RC/CS_0, φP_0, $PI_{(abs)}$, and φE_0. For this treatment (IC), clones 104A and 16 had the highest values of the Chl*a*F parameters, followed by 02, 03, 19, GG, 109A, and 73 (Table 6S, *supplement*).

For winter, principal component analysis of Chl*a*F showed a total variance of 78.9% (Fig. 3*D*). PC1 explained

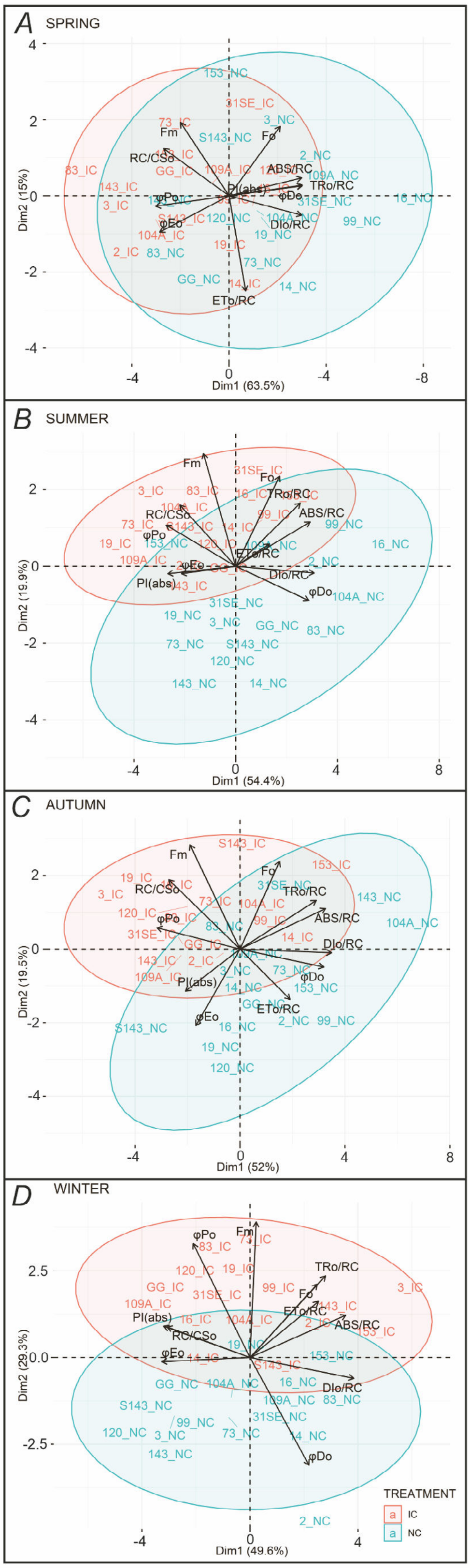

Fig. 3. Principal component analysis of JIP-test parameters of *Coffea canephora* intercropped (IC) and non-intercropped (NC) in spring (*A*), summer (*B*), autumn (*C*), and winter (*D*), with the *R* software, *version 4.1.2*. $n = 20$.

49.6% of the data variation. Clones 14, 83, 31SE, 109A, 73, 104A, GG, 99, 03, 143, 120, and S143 formed a cluster for the NC treatment. However, in this treatment, clones 14, 83, 31SE, and 109A formed a cluster. It was positioned in the lower right region of the graph (Fig. 3*D*). The formation of this cluster occurred due to significant values of the parameters φD_0 and DI_0/RC of the clones (Table 7S, *supplement*). For the IC treatment, there was a cluster in the upper right region of clones 03, 153, 143, 99, and 73, and the parameters that explained this arrangement were F_m, F_0, TR_0/RC, ET_0/RC, and ABS/RC. Clone 03 had the highest mean, followed by 143, 99, 73, and 153 (Table 7S). PC2 was responsible for 29.3% of the data variation (Fig. 3*D*), with a cluster consisting of clones 73, 104A, GG, 99, 03, 143, 120, and S143 in the NC treatment. These clones had lower averages of two nonphotochemical parameters (φD_0 and DI_0/RC) and absence of significant differences with the other parameters, explaining the formation of this cluster. For the IC treatment, there was a group located in the upper left region composed of clones 19, 83, 31SE, 120, GG, and 109A, with influence of the parameters φP_0, $PI_{(abs)}$, RC/CS_0, and φE_0, for which higher averages were obtained (Table 8S, *supplement*).

Finally, we performed PCA of the four seasons of the year together for Chl*a*F, yield, and leaf chemical data to observe the clustering pattern of the IC and NC treatments (Fig. 4). At first, we observed direct correlation for the Chl*a*F parameters (φP_0, RC/CS_0, and F_m) with productivity (PR), yield (YLD) and leaf nutrients (Mn, Cu, Zn, B, Co, Mg, and K) for the IC treatment. On the other hand, for the NC treatment, the parameters that presented the highest correlations and followed a similar distribution pattern were ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC, φD_0, P, Fe, and N. The variations observed in the parameters Chl*a*F (JIP-test), yield, and leaf chemical data for both treatments presented through the principal component analysis are corroborated by the statistical tests reported in the supplementary materials (Tables 1S–8S).

Leaf chemical analysis: For leaf chemical analysis, a few clones showed significant statistical differences for macronutrient concentration, among them 16, 99, 120, and 109A (Table 1). Clone 16 only differed statistically in the case of Ca content, with higher values obtained in the IC treatment. Clone 99 differed statistically for P and Ca, with a higher concentration of P in the NC treatment and a higher concentration of Ca in the IC treatment. The only statistical difference observed for clone 120 was related to the Mg content, with the highest average being obtained in the NC treatment. For clone 109A, all means had higher values for the intercropped treatment, with statistically significant differences for the concentrations of N and P. There was no statistically significant difference in the micronutrient K between the clones and treatments evaluated (Table 1).

Most of the clones showed statistically significant differences in the foliar concentration of micronutrients, with higher averages in the intercropped treatment, with the exception of 14, 31SE, and 153 (Table 2). The concentrations of B and Fe did not differ between

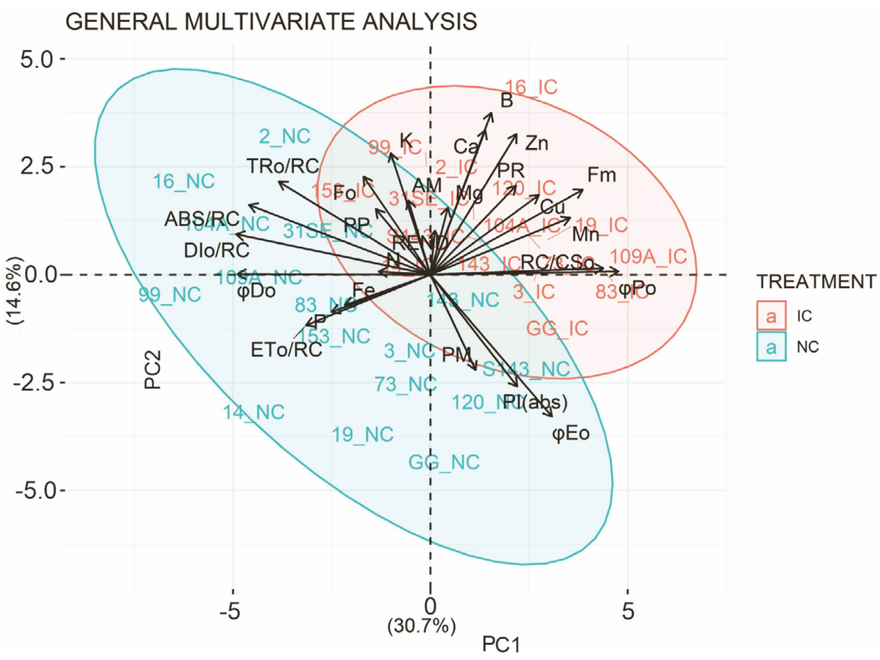

Fig. 4. Principal components analysis of JIP-test parameters, foliar nutrient, and productivity of *Coffea canephora* for intercropped (IC) and non-intercropped (NC) treatments in all seasons with the *R* software, *version 4.1.2*.

treatments for any of the clones. The concentration of Zn was the highest for clones 19, GG, 73, 83, 120, and 104A in the IC treatment. The concentration of Mn was statistically higher in the intercropped treatment for clones 16, 83, and 109A. Among the nutrients evaluated, Cu presented the greatest number of clones with statistical differences, and all the averages between treatments presented higher values in the intercropped system (Table 2).

Productivity: Clones 02, 03, 14, 16, 19, 31SE, 73, 99, 120, 153, 104A, and 109A showed the highest productivity [kg per plant] in the IC treatment. In the NC treatment, the highest productivity was recorded for clones GG, 83, 143, and S143 (Table 3). The crushed grain mass [g] was higher for clones 02, 19, 31SE, 73, 83, 143, and 109A in the IC system. In the NC treatment, the highest means were recorded for clones 03, 16, GG, 99, 120, 153, 104A, and S143. The clones 02, 03, 16, 19, 73, 83, 99, 120, 143, 153, 104A, 109A, and S143 had higher total yield in the IC treatment. Clones 14 and 31SE had the highest total yield values in the NC system.

Discussion

This study was carried out to elucidate some of the photochemical mechanisms involved in the physiological responses of coffee clones cultivated alone (NC) and intercropped (IC) with rubber trees. In general, F_0, ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC, and φD_0 values were higher in the NC condition, following a distribution pattern during the four seasons of the year. The increases of these nonphotochemical parameters in the NC system are common in environments with high solar radiation, which reduces the photochemical performance and consequently the carbon assimilation, leading to photoinhibition (DaMatta *et al.* 2016). In this sense, coffee intercropping with rubber trees, which can reduce high daytime temperatures and high light, represents an important alternative to cope with climate change in coffee production in Southeast Brazil. Also, the results of this study provide evidence that the responses of coffee to intercropping are species-specific and often genotype-specific.

The higher F_0 values observed in clones 02 and 153 (spring), 109A (summer), 31SE, 83, 143, and 104A (autumn) in NC and for 02, 03, 73, 99, 120, 143, and S143 in IC (spring) are related to partial inhibition of RC associated with PSII, reducing the electron flux from Q_A to Q_B. This, in turn, is related to the accumulation of reduced Q_A (Chen *et al.* 2015a). In the winter (September), the minimum air temperature (T_{air}) reached 18°C, with the lowest precipitation of the year (Fig. 1), which could have influenced the increase in F_0 observed. Furthermore, the invariability of F_0 values observed in the coffee clones cultivated under NC conditions during the winter, except 109A, indicates that these clones did not suffer severe damage from low temperature compared to the coffee clones cultivated in the IC condition (Araújo *et al.* 2015). The higher production of fluorescence signals in clones 02, 73, 143, and 109A, especially in the NC system, provides evidence of higher susceptibility of the photosynthetic apparatus to strong light.

The higher sensitivity of clones 02, 73, 143, and 109A to strong light can be explained by the higher photochemical efficiency, particularly φP_0, observed in plants grown in the IC condition, in contrast to the clones cultivated in the NC condition. According to Nunes *et al.* (2021), trees with a deep root system, such as *H. brasiliensis*, can increase water availability of the soil surface layers after long drought periods, reducing the water stress of coffee plants, resulting in increases of the photochemical parameters in IC condition.

Increased values of specific energy fluxes per RC for NC plants indicated inactivation of some RCs, leading

Table 1. Leaf macronutrient concentration [g kg^{-1}] of 16 clones of *Coffea canephora* grown alone (NC) and intercropped with *Hevea brasiliensis* (IC). *Lowercase letters* compare the effect of IC and NC treatment of each clone in relation to foliar nutrients. *Capital letters* compare which clones had higher means for each treatment in relation to nutrients according to the *Scott-Knott* multiple comparison test at 5% significance. Means that differ statistically ($p<0.05$) from each other are indicated in bold.

Clone	Treatment	N	P	K	Ca	Mg
2	IC	30.823 ± 0.851^{bABC}	1.413 ± 0.202^{bB}	29.503 ± 3.732^{bAB}	17.650 ± 2.713^{bAB}	3.778 ± 0.783^{bA}
	NC	33.075 ± 2.546^{bB}	1.442 ± 0.167^{bB}	26.077 ± 2.451^{bABC}	18.550 ± 3.472^{bA}	3.257 ± 0.609^{bBC}
3	IC	30.170 ± 1.483^{bABC}	1.401 ± 0.192^{bB}	28.323 ± 3.211^{bABC}	10.637 ± 2.381^{bD}	2.471 ± 0.329^{bCD}
	NC	29.225 ± 0.490^{bB}	1.465 ± 0.177^{bB}	24.370 ± 2.813^{bABC}	9.997 ± 1.525^{bB}	2.630 ± 0.890^{bBC}
14	IC	32.515 ± 1.602^{bAB}	1.321 ± 0.164^{bB}	30.053 ± 3.591^{bA}	12.738 ± 1.705^{bCD}	2.552 ± 0.359^{bCD}
	NC	33.075 ± 2.121^{bB}	1.547 ± 0.255^{bB}	30.670 ± 1.320^{bA}	9.955 ± 1.368^{bB}	2.502 ± 0.770^{bBC}
16	IC	29.575 ± 2.143^{bABC}	1.315 ± 0.196^{bB}	29.630 ± 2.547^{bAB}	**18.192 ± 2.222^{aA}**	3.511 ± 0.659^{bABC}
	NC	$29.925 \pm 2.363\ ^{bB}$	$1.402 \pm 0.212\ ^{bB}$	$28.390 \pm 1.163\ ^{bAB}$	$14.322 \pm 1.965\ ^{bAB}$	$3.017 \pm 0.203\ ^{bBC}$
19	IC	29.120 ± 1.529^{bABC}	1.401 ± 0.114^{bB}	28.432 ± 2.876^{bABC}	13.341 ± 2.981^{bBCD}	2.905 ± 0.711^{bABCD}
	NC	29.157 ± 1.702^{bB}	1.415 ± 0.117^{bB}	24.205 ± 2.061^{bABC}	11.250 ± 1.847^{bB}	2.405 ± 0.562^{bBC}
31SE	IC	30.332 ± 1.297^{bABC}	1.265 ± 0.149^{bB}	24.827 ± 2.225^{bABCD}	14.291 ± 2.356^{bABCD}	2.603 ± 0.461^{bCD}
	NC	29.190 ± 1.275^{bB}	1.480 ± 0.192^{bB}	24.562 ± 2.287^{bABC}	14.227 ± 2.023^{bAB}	3.042 ± 0.540^{bBC}
GG	IC	33.016 ± 1.830^{bAB}	1.335 ± 0.267^{bB}	18.841 ± 2.992^{bD}	14.843 ± 3.402^{bABCD}	1.885 ± 0.254^{bD}
	NC	$31.885 \pm 2.567\ ^{bB}$	$1.470 \pm 0.172\ ^{bB}$	$17.407 \pm 1.394\ ^{bC}$	$12.210 \pm 2.065\ ^{bAB}$	$1.882 \pm 0.189\ ^{bC}$
73	IC	27.737 ± 1.773^{bBC}	1.347 ± 0.248^{bB}	27.227 ± 4.693^{bABC}	13.731 ± 2.987^{bABCD}	3.311 ± 0.623^{bABC}
	NC	27.990 ± 3.101^{bB}	1.292 ± 0.174^{bB}	23.680 ± 1.526^{bABC}	12.355 ± 1.277^{bAB}	3.507 ± 0.611^{bAB}
83	IC	33.355 ± 2.173^{bA}	1.363 ± 0.238^{bB}	22.795 ± 4.644^{bCD}	14.537 ± 2.553^{bABCD}	2.955 ± 0.567^{bABC}
	NC	34.790 ± 1.025^{bB}	1.587 ± 0.167^{bB}	22.117 ± 1.420^{bABC}	12.395 ± 1.118^{bAB}	3.442 ± 0.485^{bAB}
99	IC	29.102 ± 2.299^{bABC}	1.267 ± 0.259^{bB}	26.847 ± 2.352^{bABC}	**15.323 ± 2.132^{aABC}**	3.030 ± 0.463^{bABC}
	NC	30.065 ± 3.699^{Bb}	**1.560 ± 0.258^{aB}**	27.695 ± 2.802^{bAB}	11.245 ± 1.451^{bB}	2.885 ± 0.838^{bBC}
120	IC	30.555 ± 2.149^{bABC}	1.286 ± 0.190^{bB}	25.003 ± 3.932^{bABCD}	16.428 ± 1.122^{bABC}	3.703 ± 0.313^{bAB}
	NC	28.735 ± 1.434^{bB}	1.217 ± 0.063^{bB}	21.710 ± 3.082^{bBC}	16.247 ± 1.578^{bAB}	**4.820 ± 0.494^{aA}**
143	IC	28.910 ± 1.890^{bABC}	1.408 ± 0.202^{bB}	22.477 ± 1.564^{bCD}	13.531 ± 2.680^{bBCD}	2.696 ± 0.461^{bBCD}
	NC	28.595 ± 1.764^{bB}	1.282 ± 0.162^{bB}	21.855 ± 1.686^{bABC}	11.790 ± 1.492^{bB}	2.945 ± 0.203^{bBC}
153	IC	30.220 ± 1.261^{bABC}	1.362 ± 0.162^{bB}	23.345 ± 2.783^{bCD}	13.144 ± 1.338^{bBCD}	2.924 ± 0.568^{bABCD}
	NC	29.960 ± 0.996^{bB}	1.390 ± 0.065^{bB}	24.370 ± 3.001^{bABC}	11.252 ± 0.780^{bB}	3.000 ± 0.750^{bBC}
104A	IC	30.200 ± 2.322^{bABC}	1.280 ± 0.178^{bB}	27.088 ± 3.446^{bABC}	14.040 ± 1.338^{bABCD}	3.255 ± 0.550^{bABC}
	NC	30.975 ± 1.547^{bB}	1.257 ± 0.121^{bB}	27.950 ± 1.728^{bAB}	12.637 ± 0.439^{bAB}	3.230 ± 0.593^{bBC}
109A	IC	25.780 ± 11.811^{bC}	1.071 ± 0.535^{bB}	$27.805 \pm 10.868^{bABCD}$	16.195 ± 6.870^{bABCD}	3.088 ± 1.196^{bBCD}
	NC	**31.675 ± 1.167^{aB}**	**1.495 ± 0.166^{aB}**	26.035 ± 0.988^{bABC}	13.100 ± 2.159^{bAB}	2.510 ± 0.541^{bBC}
S143	IC	29.207 ± 1.938^{bABC}	1.422 ± 0.222^{bB}	24.928 ± 2.776^{bABCD}	14.372 ± 3.275^{bABCD}	2.775 ± 0.450^{bABCD}
	NC	27.825 ± 1.837^{bB}	1.337 ± 0.196^{bB}	23.020 ± 2.594^{bABC}	11.635 ± 1.821^{bB}	2.875 ± 0.792^{bBC}

to photoinhibition (Kalaji *et al.* 2017). In this study, the increased values of ABS/RC, TR_0/RC, and DI_0/RC observed of clones 02, 16, 99, 104A, and 109A (spring), 02, 104A, and 109A (summer), and 03, 31SE, 143, 104A, and 109A (autumn) in the NC treatment suggest the occurrence of photoinhibition (Cipriano *et al.* 2021). Furthermore, when the absorption and trapped energy flux per RC were high, the electron transport flux per RC (ET_0/RC) values also were high for some coffee clones in different seasons of the year. Also, there was an increase in the dissipated energy flux per RC (DI_0/RC), which was accompanied by higher quantum dissipation energy efficiency (φD_0), especially for the NC system. According to Wang *et al.* (2016), the increase of DI_0/RC and φD_0 values may indicate photoinhibition through the dissipation of energy trapped as heat. Thus, the NC condition increases the susceptibility of coffee clones to intense sunlight. In this study, the increase in these Chl*a*F parameters associated with nonphotochemical processes observed in some coffee clones during winter may be associated with both low temperatures and reduced rainfall, a common climate characteristic of this season in northern Espírito Santo. With lower temperatures, the plant metabolism declines due to stress caused by cold, leading to inhibition of PSII activity, as reported by Bulgari *et al.* (2019).

In all sampling periods, there was a tendency for lower production of maximum fluorescence signals (F_m) for most clones submitted to the NC condition, and an increase in F_m in the IC treatment. The decreases in F_m values indicate an increase in nonphotochemical dissipation, which reduces the plant's photochemical processes (Murchie and Lawson 2013). In our study, the lower F_m values of the coffee clones cultivated alone (02, 03, 14, 16, 19, 31SE, 73, 83, 99, 120, 143, 104A, and 109A)

Table 2. Leaf micronutrient concentration [mg kg^{-1}] of 16 clones of *Coffea canephora* grown alone (NC) and intercropped with *Hevea brasiliensis* (IC). *Lowercase letters* compare the effect of IC and NC treatment for each clone in relation to foliar nutrients. *Capital letters* compare which clones had higher means for each treatment in relation to nutrients according to the *Scott-Knott* multiple comparison test at 5% significance. Means that differ statistically ($p<0.05$) from each other are indicated in bold.

Clone	Treatment	B	Zn	Mn	Fe	Cu
2	IC	64.913 ± 9.747^{bB}	10.483 ± 1.683^{bB}	64.310 ± 22.061^{bABC}	84.018 ± 55.126^{bB}	**19.045 ± 5.344^{aA}**
	NC	66.110 ± 8.909^{bB}	9.212 ± 2.909^{bB}	54.607 ± 13.081^{bB}	74.205 ± 6.297^{bB}	12.507 ± 3.380^{bB}
3	IC	59.086 ± 7.099^{bB}	8.633 ± 3.154^{bB}	63.256 ± 22.345^{bABC}	70.721 ± 26.722^{bB}	**18.462 ± 1.790^{aA}**
	NC	56.887 ± 5.737^{bB}	5.712 ± 3.776^{bB}	50.627 ± 13.682^{bB}	66.522 ± 3.229^{bB}	14.190 ± 2.147^{bB}
14	IC	56.695 ± 4.688^{bB}	8.440 ± 2.771^{bB}	47.411 ± 10.074^{bBC}	88.983 ± 73.054^{bB}	16.181 ± 1.651^{bAB}
	NC	48.447 ± 2.626^{bB}	7.367 ± 3.569^{bB}	39.157 ± 11.341^{bB}	71.722 ± 4.283^{bB}	12.350 ± 1.200^{bB}
16	IC	66.080 ± 4.832^{bB}	**11.757 ± 2.381^{aB}**	**71.666 ± 20.554^{aABC}**	68.397 ± 14.021^{bB}	**16.515 ± 4.656^{aAB}**
	NC	57.902 ± 10.237^{bB}	6.605 ± 2.791^{bB}	43.612 ± 7.062^{bB}	73.357 ± 2.892^{bB}	11.902 ± 1.797^{bB}
19	IC	65.091 ± 6.310^{bB}	6.843 ± 2.946^{bB}	59.420 ± 22.472^{bBC}	63.148 ± 10.038^{bB}	**18.992 ± 2.783^{aA}**
	NC	52.755 ± 5.456^{bB}	5.470 ± 1.637^{bB}	39.260 ± 11.091^{bB}	75.982 ± 7.218^{bB}	12.512 ± 2.439^{bB}
31SE	IC	59.922 ± 8.071^{bB}	8.427 ± 2.546^{bB}	43.335 ± 13.286^{bC}	70.607 ± 14.003^{bB}	15.418 ± 2.548^{bAB}
	NC	57.345 ± 6.335^{bB}	6.290 ± 0.965^{bB}	51.555 ± 16.134^{bB}	72.862 ± 2.973^{bB}	13.767 ± 3.581^{bB}
GG	IC	53.720 ± 3.290^{bB}	**9.630 ± 1.911^{aB}**	61.903 ± 13.560^{bABC}	66.116 ± 12.933^{bB}	**19.143 ± 5.442^{aA}**
	NC	46.860 ± 7.934^{bB}	5.375 ± 3.126^{bB}	58.840 ± 9.098^{bB}	78.520 ± 9.225^{bB}	13.535 ± 2.535^{bB}
73	IC	53.971 ± 6.489^{bB}	**9.780 ± 3.251^{aB}**	68.736 ± 15.908^{bABC}	65.100 ± 5.362^{bB}	**16.500 ± 3.238^{aAB}**
	NC	46.180 ± 5.123^{bB}	3.682 ± 2.042^{bB}	64.567 ± 16.524^{bB}	68.462 ± 7.865^{bB}	9.037 ± 1.724^{bB}
83	IC	59.936 ± 7.487^{bB}	**9.541 ± 2.372^{aB}**	**97.253 ± 22.382^{aA}**	73.316 ± 14.920^{bB}	14.206 ± 4.030^{bAB}
	NC	54.060 ± 3.431^{bB}	6.142 ± 3.076^{bB}	64.192 ± 7.355^{bB}	98.020 ± 62.231^{bB}	13.645 ± 2.633^{bB}
99	IC	60.882 ± 7.168^{bB}	8.908 ± 2.347^{bB}	57.598 ± 12.023^{bBC}	62.682 ± 8.388^{bB}	**14.652 ± 2.248^{aAB}**
	NC	58.127 ± 6.326^{bB}	7.320 ± 2.201^{bB}	49.820 ± 9.009^{bB}	86.195 ± 52.553^{bB}	10.460 ± 2.948^{bB}
120	IC	59.588 ± 5.438^{bB}	**8.932 ± 2.241^{aB}**	67.782 ± 9.394^{bABC}	62.212 ± 5.283^{bB}	**14.772 ± 3.360^{aAB}**
	NC	52.840 ± 4.838^{bB}	3.267 ± 2.375^{bB}	61.172 ± 15.537^{bB}	71.010 ± 1.464^{bB}	9.857 ± 1.920^{b}
143	IC	62.567 ± 7.939^{bB}	8.927 ± 1.237^{bB}	76.096 ± 31.371^{bABC}	58.842 ± 7.926^{bB}	**13.968 ± 3.800^{aAB}**
	NC	58.950 ± 5.835^{bB}	8.722 ± 2.863^{bB}	64.455 ± 2.221^{bB}	66.705 ± 3.512^{bB}	9.625 ± 5.061^{bB}
153	IC	57.822 ± 7.481^{bB}	7.724 ± 3.115^{bB}	67.545 ± 21.776^{bABC}	63.191 ± 6.803^{bB}	**16.455 ± 3.637^{aAB}**
	NC	48.032 ± 2.081^{bB}	6.115 ± 3.941^{bB}	45.867 ± 5.560^{bB}	64.540 ± 5.004^{bB}	9.477 ± 1.207^{bB}
104A	IC	52.827 ± 6.956^{bB}	**8.972 ± 2.964^{aB}**	77.530 ± 22.214^{bABC}	60.368 ± 5.594^{bB}	**13.710 ± 2.381^{aAB}**
	NC	46.277 ± 2.241^{bB}	4.020 ± 1.229^{bB}	58.907 ± 8.524^{bB}	67.375 ± 6.015^{bB}	7.167 ± 0.867^{bB}
109A	IC	63.465 ± 25.853^{bB}	10.526 ± 4.444^{bB}	**86.460 ± 56.104^{aAB}**	76.106 ± 36.781^{bB}	14.076 ± 6.469^{bB}
	NC	56.235 ± 6.864^{bB}	8.515 ± 3.234^{bB}	40.177 ± 4.959^{bB}	80.862 ± 8.393^{bB}	11.180 ± 1.800^{bB}
S143	IC	59.150 ± 7.883^{bB}	8.455 ± 2.391^{bB}	67.291 ± 23.457^{bABC}	65.388 ± 12.398^{bB}	**15.325 ± 3.201^{aAB}**
	NC	56.167 ± 7.420^{bB}	6.095 ± 5.275^{bB}	46.785 ± 12.867^{bB}	75.380 ± 9.657^{bB}	10.697 ± 1.652^{bB}

reflect partial suppression of the OJIP transients, which is considered a good indication of stress. Generally, light stress leads to inhibition of the plastoquinone pool (PQ) and electron acceptors associated with PSI, as verified by Martins *et al.* (2015). F$_m$ is reached when all reaction centers (RCs) are closed, and all electron acceptors are reduced (Kalaji *et al.* 2017).

In contrast, the higher F$_m$ observed in coffee trees is associated with greater efficiency of PQ reduction, with consequent lower energy lost as heat (lower nonphotochemical dissipation), which increases the protection of PSII against photoinhibition and reduces the oxidative stress (Kalaji *et al.* 2017). In the IC condition, there was an increase ($p \leq 0.05$) of Q$_A$-reducing RCs (RC/CS$_0$), of maximum quantum yield for PSII primary photochemistry (φP_0), and of efficiency/probability that an electron moves further than Q$_A$ (φE_0). These results indicate that the shade induces a redox reaction after Q$_A$, which improves the electron transfer between Q_A^- and Q_B^- (Lotfi *et al.* 2018). According to Kalaji *et al.* (2017), higher PI$_{(abs)}$ values are an indication of better performance of photochemical processes associated with PSII, especially for clones 02, 73, and 109A. The higher PI$_{(abs)}$ values associated with higher F$_m$, RC/CS$_0$, φP_0, and φE_0 show the importance of intercropping with other tree species to improve the microclimatic conditions of coffee plantations, with a positive effect by reducing intense radiation, and consequent indirect effects on availability of water in the soil and atmosphere for specific coffee genotypes (Piato *et al.* 2020).

Therefore, the decreases of RC/CS$_0$, φP_0, φE_0, and PI$_{(abs)}$ values in clones 02, 03, 19, 73, 83, 99, and 109A cultivated in the NC condition reveal an imbalance in the process involving light absorption by the photosynthetic

Table 3. Productivity parameters of the 16 *Coffea canephora* clones grown alone (NC) and intercropped with *Hevea brasiliensis* (IC). *Lowercase letters* compare the effect of IC and NC treatments for each clone in relation to productivity parameters. *Capital letters* compare which clones have higher means for each treatment in relation to the parameters according to the *Scott-Knott* multiple comparison test at 5% significance. Means that differ statistically ($p<0.05$) from each other are indicated in bold.

Clone	Treatment	Productivity [kg]	Crushed grain weight [g]	Total yield [kg]
2	IC	**33.178 ± 8.965^{aD}**	**326.500 ± 85.811^{aC}**	**4.217 ± 0.497^{aL}**
	NC	32.550 ± 18.940^{bC}	273.000 ± 77.326^{bH}	4.120 ± 0.357^{bH}
3	IC	**28.631 ± 6.045^{aG}**	260.500 ± 46.850^{bJ}	**4.326 ± 0.204^{aH}**
	NC	23.375 ± 11.855^{bJ}	**269.250 ± 64.680^{aJ}**	4.182 ± 0.262^{bG}
14	IC	**14.971 ± 3.311^{aP}**	**225.250 ± 7.932^{aP}**	4.444 ± 0.154^{bE}
	NC	9.750 ± 4.305^{bP}	222.750 ± 7.932^{bN}	**4.504 ± 0.162^{aD}**
16	IC	**38.671 ± 9.480^{aB}**	311.250 ± 36.160^{bD}	**4.421 ± 0.439^{aF}**
	NC	28.412 ± 8.962^{bE}	**471.000 ± 30.190^{aB}**	3.715 ± 0.525^{bK}
19	IC	**27.921 ± 4.669^{aL}**	**337.250 ± 61.021^{aA}**	**4.080 ± 0.054^{aM}**
	NC	16.220 ± 8.481^{bN}	277.250 ± 78.295^{bF}	3.610 ± 0.873^{bN}
31SE	IC	**27.271 ± 7.249^{aK}**	**295.000 ± 73.152^{aE}**	4.237 ± 0.272^{bK}
	NC	23.387 ± 5.439^{bI}	179.887 ± 11.879^{bP}	**5.586 ± 0.249^{aA}**
GG	IC	17.681 ± 5.899^{bO}	246.750 ± 55.289^{bO}	4.563 ± 0.239^{bC}
	NC	**22.462 ± 11.935^{aK}**	**287.000 ± 13.612^{aC}**	**4.790 ± 0.286^{aC}**
73	IC	**48.356 ± 7.457^{aA}**	**265.250 ± 50.585^{aH}**	**4.245 ± 0.126^{aJ}**
	NC	36.225 ± 6.813^{bB}	251.500 ± 5.972^{bL}	3.984 ± 0.094^{bJ}
83	IC	36.237 ± 6.457^{bC}	**250.500 ± 69.538^{aN}**	**5.000 ± 0.449^{aA}**
	NC	**44.825 ± 7.702^{aA}**	202.500 ± 18.627^{bO}	4.950 ± 0.454^{bB}
99	IC	**28.931 ± 9.117^{aF}**	263.750 ± 55.289^{bI}	**4.277 ± 0.049^{aI}**
	NC	12.387 ± 6.217^{bO}	**284.750 ± 38.395^{aD}**	3.499 ± 0.509^{bP}
120	IC	**28.275 ± 16.600^{aH}**	251.500 ± 39.803^{bM}	**4.482 ± 0.260^{aD}**
	NC	24.412 ± 11.000^{bF}	**261.750 ± 56.346^{aK}**	4.310 ± 0.397^{bF}
143	IC	23.809 ± 4.998^{bM}	**328.750 ± 86.318^{aB}**	**3.810 ± 0.152^{aO}**
	NC	**29.825 ± 2.708^{aD}**	275.500 ± 13.178^{bG}	3.636 ± 0.173^{bM}
153	IC	**27.921 ± 5.380^{aI}**	277.000 ± 12.569^{bF}	**3.610 ± 0.158^{aP}**
	NC	20.815 ± 0.811^{bL}	**281.250 ± 14.384^{aE}**	3.505 ± 0.001^{bO}
104A	IC	**26.981 ± 8.026^{aL}**	255.000 ± 59.155^{bK}	**4.411 ± 0.155^{aG}**
	NC	23.800 ± 8.96^{bH}	**500.250 ± 24.849^{aA}**	4.000 ± 0.244^{bI}
109A	IC	**32.381 ± 2.401^{aE}**	**266.250 ± 54.481^{aG}**	**4.699 ± 0.239^{aB}**
	NC	17.087 ± 11.106^{bM}	228.250 ± 13.047^{bM}	4.385 ± 0.257^{bE}
S143	IC	20.921 ± 3.312^{bN}	254.500 ± 5.000^{bL}	**3.937 ± 0.076^{aM}**
	NC	**23.937 ± 12.068^{aG}**	**270.000 ± 20.346^{aI}**	3.703 ± 0.280^{bL}

apparatus (Kalaji *et al.* 2017). This is corroborated by the decrease reported in Q_A-reducing RCs per CS (RC/CS$_0$). Unfortunately, these results indicate loss of plant photosynthetic performance, mainly resulting from the decreased electron acceptor pool and reaction centers associated with PSII, which consequently reduces the performance index (potential) of energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors [PI$_{(abs)}$], which is closely associated with lower energy conservation (Kalaji *et al.* 2017, Wu *et al.* 2020).

The reductions observed in φP_0 and φE_0 in clones 02, 03, 19, 73, 83, 99, and 109A for plants grown in the NC condition support the results previously described. According to Kalaji *et al.* (2017), reduced φP_0 and φE_0 values indicate an imbalance of light absorption, compromising photosynthetic processes. In the NC system, the impaired photosynthetic efficiency was corroborated by the decreased Q_A-reducing RCs per CS (RC/CS$_0$). These results indicate a loss of photosynthetic activity, mainly resulting from the decreased pool of electron acceptors and reaction centers associated with PSII, thus reducing the performance index of energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors [PI$_{(abs)}$], which is closely related to a loss of energy conservation (Kalaji *et al.* 2017, Wu *et al.* 2020).

In general, the response pattern observed for all seasons, which grouped photochemical parameters for coffee clones grown in IC and NC conditions, is closely related to the origin of the species *Coffea canephora*, which is native to tropical forests on the African continent, occurring spontaneously as understory vegetation (Verleysen *et al.* 2024). This natural occurrence of conilon in shaded habitats explains the results obtained in this study, at least for some of the clones evaluated. Thus, the high values of photochemical parameters obtained for some coffee clones cultivated under *Hevea brasiliensis* trees (02, 03, 16, 19, 73, 99, 120, 143, 104A, and 109A) show that intercropping with other species improves the photochemical process of coffee plants. In contrast, the coffee clones cultivated under NC conditions had high photochemical performance in full sunlight, making them more suitable for monocropping.

When multivariate analysis was performed using all the data, we observed a strong correlation between Chl*a*F parameters and productivity, as well as with Mg, Ca, Zn, Cu, and Mn contents in the IC condition (*see* Fig. 4). The high concentration of these mineral nutrients in the IC condition occurs due to the leaf litter accumulated on the ground from rubber trees. According to Froufe *et al.* (2020), the combination of species results in higher organic matter cycling, forming a natural fertilizer for plants cultivated under shade. Thus, the organic matter cycling reduces the requirement for fertilizers, especially nitrogen (Celi *et al.* 2022), improving the photosynthetic performance of plants, as observed by the increment in F_m, RC/CS_0, and φP_0 values in the IC condition. Comparing shaded systems with coffee grown under full sunlight, Araújo *et al.* (2016) obtained similar results for Mn but similar concentrations of Mg, Ca, Zn, and Cu, thereby contradicting, in part, the results observed in this study. Mn^{2+} activates plant enzymes of the Krebs cycle (such as decarboxylases and dehydrogenases) as well as constituting the oxygen-evolving complex (OEC) associated with PSII through which oxygen (O_2) is produced from water. According to Najafpour *et al.* (2014), the OEC is a manganese-calcium $[Mn_4CaO_5(H_2O)_4]$ cluster stored in a protein complex.

In the NC area, we observed significant increases in P and N, mainly of clones 99 and 109A (Table 1). Nitrogen is one of the most required nutrients for plant growth and development. N is a constituent of amino acids, proteins, cell walls, membranes, and nucleic acids (Marschner 2012). Thus, N is closely related to the productivity of coffee plantations, being required for chlorophyll synthesis (de Souza *et al.* 2020). Low N concentrations reduce photosynthesis and leaf area, impairing plant growth and development, and thus productivity (Chen *et al.* 2015b, Mu *et al.* 2017). Phosphorus, in turn, plays key roles in regulating energy metabolism, the synthesis of nucleic acids, and membranes. P is present in compounds such as ATP, NADPH, and phospholipids, which play important roles in photosynthesis (Bisson *et al.* 2017). P deficiency reduces the root and shoot system development of coffee trees and lowers yield (Bernardino *et al.* 2019, Epie *et al.* 2019).

The increase in total yield observed in the 13 coffee clones cultivated in the IC system corroborates the higher photochemical efficiency and better leaf chemical parameters. In this study, total yield was the parameter that most influenced productivity, and the clones 02, 03, 16, 19, 73, 83, 99, 120, 143, 153, 104A, 109A, and S143 were most suitable for IC treatment with rubber trees (Table 2). In studies with intercropped coffee, benefits in productivity and bean quality have been reported (Machado Filho *et al.* 2024). Assis *et al.* (2019), in a study with four coffee varieties, observed that the number of fruits per plant increased in response to shade levels among all coffee varieties tested, suggesting the possibility of achieving better productive results with higher levels of shade. The study demonstrated that, when shaded, coffee trees showed greater production, which is an alternative to reduce production costs, resulting in diversification when they are in an agroforestry system. In Mexico, Yuliasmara *et al.* (2022) did not observe a reduction in coffee productivity per plant, up to a limit of 50% shade. The authors concluded that the use of shade would be the best alternative for small farmers in the region, with limited investment capacity and family labor.

The occurrence of light stress in clones 03, 31SE, 143, 104A, 109A, and 99 under the NC condition was revealed by the increased nonphotochemical parameters associated with the increment of both some leaf nutrients and yield of clones 14, 31SE, and GG in the NC area. This suggested a higher tolerance of these coffee clones to intense light. Considering this division of clones into those grown with shade (IC) and in full sunlight (NC), Machado Filho *et al.* (2024) suggested investigating the direction of planting rows of coffee trees in relation to the rows of rubber trees. Thus, the promising coffee clones for shaded growth would be arranged as close to the shadow projection of the rubber trees, while the clones showing better performance under NC conditions would be arranged in the central area, receiving stronger luminosity.

Conclusion: In conclusion, the results of this study support the hypothesis that shade provided by *H. brasiliensis* and the exposure of coffee clones to full sunlight would modulate the physiology of the latter plants detected through Chl*a*F measurements, leaf chemical parameters, and clone productivity in both IC and NC treatments. In full sunlight, clones 14, 31SE, and GG suffered photoinhibitory damage in the PSII complex. However, even under stress, these clones achieved greater productivity, leading us to categorize them as beneficiaries of greater exposure to light. In the IC treatment, clones 02, 03, 16, 19, 73, 83, 99, 120, 143, 153, 104A, 109A, and S143 showed positive modulation of photochemical and nutritional activity, reflecting greater productivity. The higher productivity of IC clones categorizes them as beneficiaries of the combination of coffee with rubber trees. These results are extremely important for coffee production because they provide a better understanding of coffee's behavior in response to intercropping, as well as optimization of management in order to improve physiological processes, resulting in higher productivity.

References

ABIC – Brazilian Coffee Industry Association: Coffee Industry Indicators – Consumption Statistics in 2020, 2021. *Available at*: https://www.abic.com.br/.

Araújo A.V., Partelli F.L., Oliosi G., Pezzopane J.R.M.: Microclimate, development and productivity of robusta coffee shaded by rubber trees and at full sun. – Rev. Ciênc. Agron.

47: 700-709, 2016.

Araújo A.V., Partelli F.L., Oliveira M.G. *et al.*: [Microclimatic and vegetative growth in coffee and banana intercrop.] – Coffee Sci. **10**: 214-222, 2015. [In Portuguese]

Assis B.D.P., Gross E., Pereira N.E. *et al.*: Growth response of four conilon coffee varieties (*Coffea canephora* Pierre ex A. Froehner) to different shading levels. – J. Agr. Sci. **11**: 29-41, 2019.

Bernardino K.C., Pastina M.M., Menezes C.B. *et al.*: The genetic architecture of phosphorus efficiency in sorghum involves pleiotropic QTL for root morphology and grain yield under low phosphorus availability in the soil. – BMC Plant Biol. **19**: 87, 2019.

Bisson C., Adams N.B.P., Stevenson B. *et al.*: The molecular basis of phosphite and hypophosphite recognition by ABC-transporters. – Nat. Commun. **8**: 1746, 2017.

Bulgari R., Franzoni G., Ferrante A.: Biostimulants application in horticultural crops under abiotic stress conditions. – Agronomy **9**: 306, 2019.

Carmo C.A.F., Araujo W.S., Bernardi A.C., Saldanha M.F.C.: [Plant tissue analysis methods used at Embrapa Solos.] Pp. 41. Embrapa Solos, Rio de Janeiro 2000. [In Portuguese]

Celi L., Said-Pullicino D., Bol R. *et al.*: Interconnecting soil organic matter with nitrogen and phosphorus cycling. – In: Yang Y., Keiluweit M., Senesi N., Xing B. (ed.): Multi-Scale Biogeochemical Processes in Soil Ecosystems: Critical Reactions and Resilience to Climate Changes. Pp. 51-77. John Wiley & Sons, Inc., Hoboken 2022.

Chen Q., Guan T., Yun L. *et al.*: Online forecasting chlorophyll *a* concentrations by an auto-regressive integrated moving average model: feasibilities and potentials. – Harmful Algae **43**: 58-65, 2015a.

Chen Y., Xiao C., Wu D. *et al.*: Effects of nitrogen application rate on grain yield and grain nitrogen concentration in two maize hybrids with contrasting nitrogen remobilization efficiency. – Eur. J. Agron. **62**: 79-89, 2015b.

Cipriano R., Martins J.P.R., Rodrigues L.C.D.A. *et al.*: Impact of saline solution on growth and photosystem II during *in vitro* cultivation of *Bromelia antiacantha* (Bromeliaceae). – Rodriguésia **72**: e01242019, 2021.

CONAB – The National Supply Company: Monitoring of the Brazilian Coffee Crop, 2021. *Available at*: https://portaldeinformacoes.conab.gov.br/produtos-360.html.

DaMatta F.M., Godoy A.G., Menezes-Silva P.E. *et al.*: Sustained enhancement of photosynthesis in coffee trees grown under free-air CO_2 enrichment conditions: disentangling the contributions of stomatal, mesophyll, and biochemical limitations. – J. Exp. Bot. **67**: 341-352, 2016.

de Souza B.P., Martinez H.E.P., de Carvalho F.P. *et al.*: Gas exchanges and chlorophyll fluorescence of young coffee plants submitted to water and nitrogen stresses. – J. Plant Nutr. **43**: 2455-2465, 2020.

Epie K.E., Etesami M., Ondoua R.N.: Characterization and selection for phosphorus deficiency tolerance in 99 spring wheat genotypes in Montana. – J. Plant Nutr. **42**: 595-603, 2019.

Ferreira D.S., de Sales R.A., Amaral J.F.T.D. *et al.*: Biochemical and physiological changes in conilon coffee (*Coffea canephora*) grown under different levels of irradiance. – Aust. J. Crop Sci. **15**: 1074-1080, 2021.

Froufe L.C.M., Schwiderke D.K., Castilhano A.C. *et al.*: Nutrient cycling from leaf litter in multistrata successional agroforestry systems and natural regeneration at Brazilian Atlantic Rainforest Biome. – Agroforest. Syst. **94**: 159-171, 2020.

Gill S.S., Tuteja N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – Plant Physiol. Biochem. **48**: 909-930, 2010.

Gomes L.C., Bianchi F.J.J.A., Cardoso I.M. *et al.*: Agroforestry systems can mitigate the impacts of climate change on coffee production: a spatially explicit assessment in Brazil. – Agr. Ecosyst. Environ. **294**: 106858, 2020.

ICO – International Coffee Organization: Anual Review: Coffee Year 2021/2022. Pp. 32. ICO, London 2022. *Available at*: https://www.icocoffee.org/documents/cy2022-23/annual-review-2021-2022-e.pdf.

Kalaji H.M., Schansker G., Brestic M. *et al.*: Frequently asked questions about chlorophyll fluorescence, the sequel. – Photosynth. Res. **132**: 13-66, 2017.

Lorençone P.A., de Oliveira Aparecido L.E., Lorençone J.A. *et al.*: Climate change and its consequences on the climatic zoning of *Coffea canephora* in Brazil. – Environ. Dev. Sustain. **26**: 8377-8398, 2024.

Lotfi R., Kalaji H.M., Valizadeh G.R. *et al.*: Effects of humic acid on photosynthetic efficiency of rapeseed plants growing under different watering conditions. – Photosynthetica **56**: 962-970, 2018.

Machado Filho J.A., Costa P.R., Arantes L.O. *et al.*: Yield and beverage quality of thirty-one *Coffea canephora* clones shaded by *Hevea brasiliensis*. – Coffee Sci. **19**: e192259, 2024.

Marschner P.: Marschner's Mineral Nutrition of Higher Plants. Pp. 651. Academic Press, Amsterdam 2012.

Martins J.P.R., Schimildt E.R., Alexandre R.S. *et al.*: Chlorophyll *a* fluorescence and growth of *Neoregelia concentrica* (Bromeliaceae) during acclimatization in response to light levels. – In Vitro Cell. Dev.-Pl. **51**: 471-481, 2015.

Medauar C.C., Silva S.D.A., Galvão Í.M. *et al.*: Fuzzy mapping of climate favorability for the cultivation of conilon coffee in the state of Bahia, Brazil. – Int. J. Fruit Sci. **21**: 205-217, 2021.

Mu X., Chen Q., Chen F. *et al.*: A RNA-Seq analysis of the response of photosynthetic system to low nitrogen supply in maize leaf. – Int. J. Mol. Sci. **18**: 2624, 2017.

Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – J. Exp. Bot. **64**: 3983-3998, 2013.

Najafpour M.M., Isaloo M.A., Eaton-Rye J.J. *et al.*: Water exchange in manganese-based water-oxidizing catalysts in photosynthetic systems: from the water-oxidizing complex in photosystem II to nano-sized manganese oxides. – BBA-Bioenergetics **1837**: 1395-1410, 2014.

Nunes A.L.P., Cortez G.L.S., Zaro G.C. *et al.*: Soil morphostructural characterization and coffee root distribution under agroforestry system with *Hevea brasiliensis*. – Sci. Agric. **78**: e20190150, 2021.

Partelli F.L., Marré W.B., Falqueto A.R. *et al.*: Seasonal vegetative growth in genotypes of *Coffea canephora*, as related to climatic factors. – J. Agr. Sci. **5**: 108-116, 2013.

Peloso A.F., Tatagiba S.D., Amaral J.F.T. *et al.*: [Effect of the application of pyraclostrobin in the initial growth of arabic coffee in different availability of water.] – Coffee Sci. **12**: 498-507, 2017. [In Portuguese]

Piato K., Lefort F., Subía C. *et al.*: Effects of shade trees on robusta coffee growth, yield and quality. A meta-analysis. – Agron. Sustain. Dev. **40**: 38, 2020.

R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2020. *Available at*: https://www.R-project.org.

Rodrigues W.P., Vieira H.D., Campostrini E. *et al.*: Physiological aspects, growth and yield of *Coffea* spp. in areas of high altitude. – Aust. J. Crop Sci. **10**: 666-674, 2016.

Semedo J.N., Rodrigues W.P., Dubberstein D. *et al.*: Coffee responses to drought, warming and high [CO_2] in a context of future climate change scenarios. – In: Alves F., Leal Filho W., Azeiteiro U. (ed.): Theory and Practice of Climate Adaptation. Climate Change Management. Pp. 465-477. Springer, Cham 2018.

Strasser B.J., Strasser R.J.: Measuring fast fluorescence transients to address environmental questions: The JIP-test. – In: Mathis P. (ed.): Photosynthesis: From Light to Biosphere. Vol. 5. Pp. 977-980. Kluwer Academic Publishers, Dordrecht 1995.

Verleysen L., Depecker J., Bollen R. *et al.*: Crop-to-wild gene flow in wild coffee species: the case of *Coffea canephora* in the Democratic Republic of the Congo. – Ann. Bot.-London **133**: 917-930, 2024.

Wang Y.W., Xu C., Lv C.F. *et al.*: Chlorophyll *a* fluorescence analysis of high-yield rice (*Oryza sativa* L.) LYPJ during leaf senescence. – Photosynthetica **54**: 422-429, 2016.

Wu H.Y., Dong F.Q., Liu L.A. *et al.*: Dorsoventral variation in photosynthesis during leaf senescence probed by chlorophyll *a* fluorescence induction kinetics in cucumber and maize plants. – Photosynthetica **58**: 479-487, 2020.

Yuliasmara F., Sumirat U., Wicaksono K.P., Widaryanto E.: Growth and plant architecture of several introduced *Coffea canephora* clones under different shade levels. – Pelita Perkebunan **38**: 155-170, 2022.