Labor & Engenho

Effect of elevated atmospheric CO_2 on spectral reflectance of coffee leaves of plants cultivated at FACE facility

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Monitoramento, relato e verificação da agricultura de baixa emissão de carbono em processos de uso e cobertura da terra

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ABSTRACT

Climate change impacts are stressing many economic sectors worldwide, including agriculture, increasingly hindering efforts to meet human needs. Dioxide carbon is one of the main greenhouse gases and it affects directly the crop production. The objective of this study was to evaluate if established remote sensing indices could detect the effects of elevated atmospheric CO_2 on the leaves of coffee (*Coffea arabica* L.) plantation growing under field conditions. Plots of coffee plants were exposed to ambient air (~390 μmol CO_2 mol-1) and elevated CO_2 (~550 μmol CO_2 mol-1) at the free air CO_2 enrichment (FACE) experiment. The statistical design was the completely randomized blocks with six replicates per treatment (ambient CO_2 and elevated CO_2), with 10m-diameter plots. Coffee leaves were spectrally characterized by reflectance spectra on their adaxial surfaces and seven vegetation indices were calculated from reflectance data: chlorophyll normalized difference index (Chl NDI), normalized difference nitrogen index (NDNI), normalized difference vegetation index (NDVI), photochemical reflectance index (PRI), pigment specific simple ration indices for chlorophyll a (PSSRa) and chlorophyll b (PSSRb), and structural independent pigment index (SIPI). NDNI was a sensitive indicator of the atmospheric CO_2 effects on coffee leaves. NDVI, PSSRa and PSSRb were sensitive to estimate the effect of elevated CO_2 only under drought conditions. These indices identify the effect of CO_2 when a long period with high precipitation deficit stressing the leaves occurred. Chl NDI, PRI and SIPI were not sensitive to atmospheric CO_2.

KEYWORDS

CO_2. *Coffea arabica* L.. Spectral index. FACE.

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1. Introduction

Since around 1750, greenhouse gas concentrations have continued to increase in the atmosphere unequivocally caused by human activities; and each of the last four decades has been successively warmer than any decade that preceded it since 1850 (IPCC, 2021). However, an increase of carbon dioxide (CO_2) increases plant photosynthetic activity, and consequently improves agricultural productivity; but the magnitude of such benefit and the real meaning of the CO_2 interaction with other biotic and environmental factors still need to be well understood (Porter et al., 2014). For example, recent investigations have been carried out on how elevated CO_2 negatively impact on nutritional value depending on crop species and cultivars, which has important implications for human nutrition (Smith & Myers, 2018, Ainsworth & Long, 2021).

Studies under controlled conditions might not reflect responses in field conditions, where countless interactions among environmental variables may occur, and so, FACE facilities offer more realistic options to understand how increasing atmospheric CO_2 content can influence plant performance in terms of growth, yield, and pest responses (Ghini et al., 2015). Rising atmospheric CO_2 concentration, in FACE experiments, had the potential to positively impact C3 food crop production by directly stimulating photosynthetic carbon gain, which leaded to increased crop biomass and yield (Bishop, Leakey, & Ainsworth, 2014), but under nitrogen deficiency and wet conditions the yield responses of C3 crops were diminished, and for C4 crops, they would not be more productive in elevated CO_2, except under drought condition (Ainsworth & Long, 2021).

One important remote sensing approach to estimate agricultural productivity includes the spectral characterization of detached leaves (Campbell et al., 2007). From this point of view, climate change is expected to affect both morphological and physiological processes, intrinsic to the leaf development. Additionally, the leaf pigmentation and leaf internal structure could be affected by atmospheric CO_2 concentrations; and changes in the spectral properties of detached leaves are probably associated to changes in their internal structure under higher atmospheric CO_2 concentrations and environmental temperatures (Carter, Bahadur & Norby, 2000). Leaf reflectance increases in some tropical species under similar environmental conditions (Carter, Bahadur & Norby, 2000, Thomas, 2005).

A study in FACE facility evaluated the spectral reflectance from soybean canopy exposed to elevated CO_2 and concluded that reflectance data, although less sensitive than direct measurements of physiological/structural parameters, corroborated direct measurements of leaf area index, demonstrating that spectral indices accurately represented structural and physiological effects of changing tropospheric chemistry on soybean growing in a field setting (Gray, Dermody & DeLucia, 2010).

Data from experimental studies testing how species respond to each climate factor provide information on the fundamental responses to climate and atmospheric conditions to the projecting future climate impacts modelling. Experimental studies in a free-air carbon dioxide enrichment (FACE) facility allow the exposition of plants to elevated CO_2 atmospheric concentrations testing how species respond to this factor under field conditions. Remote sensing allows rapid and non-invasive estimation of physiological parameters which are important to determining productivity and is an excellent tool for large-scale assessment of ecosystem structure and function; however, it is crucial to verify whether reflectance indices calculated from remote sensing measurements have the accuracy and sensitivity to detect the effects of elevated CO_2 on ecosystem structure and function (Gray, Dermody & DeLucia, 2010). The objective of this study was to evaluate if established remote sensing indices could detect the effects of elevated atmospheric CO_2 on the leaves of coffee plantation growing under field conditions provided by FACE facility.

2. Material and Methods

The study was conducted in the FACE facilities located at Embrapa Environment (Jaguariúna, São Paulo state – 22° 43´S, 47° 01´W, 570m above sea level) during the months of April, June, August, October, and December of 2014 and 2015. The experiment was carried out in a 7ha “Catuaí” variety coffee plantation which design consisted of plantation lines 3.5m apart and 0.60m of coffee plants spaced in the line. Coffee (*Coffea arabica* L.) presents a biennial vegetative cycle in tropical condition in Brazil, and it is composed of six phenological phases (Camargo & Camargo, 2001), which were related to the data (year and the months) of field campaign.

The completely randomized blocks design with six replicates per treatment (ambient CO_2 and elevated CO_2) was adopted as experimental design, with six 10m-diameter plots (Figure 1). The blocks with two plots, ambient CO_2 and elevated CO_2, were randomly placed. Plots with elevated CO_2 and ambient CO_2 were at least 70m apart in order to minimize cross-plot contamination. Levels of CO_2 were around 550 µmol CO_2 mol-1 for elevated CO_2 and approximately 390 µmol CO_2 mol-1 in the ambient CO_2 plots (Ghini et al., 2015).

Climatically, in 2014 and early 2015, the State of Sao Paulo in Brazil suffered a major drought, with impacts in water availability for public consumption, hydropower generation, and agriculture (Coelho, Cardoso & Firpo, 2015).

The detached leaf spectral characterization was carried out through directional-hemispherical reflectance factors (reflectance), calculated on the adaxial surface of selected leaves that were detached from plants growing in the plots. From each plot, 10 selected leaves were detached once every two months, in April, June, August, October, and December of 2014 and 2015, considering the leaves from the third or fourth pair of leaves located at the plant mid height; and then transported to the laboratory inside open plastic bags during a 10-minute ride.

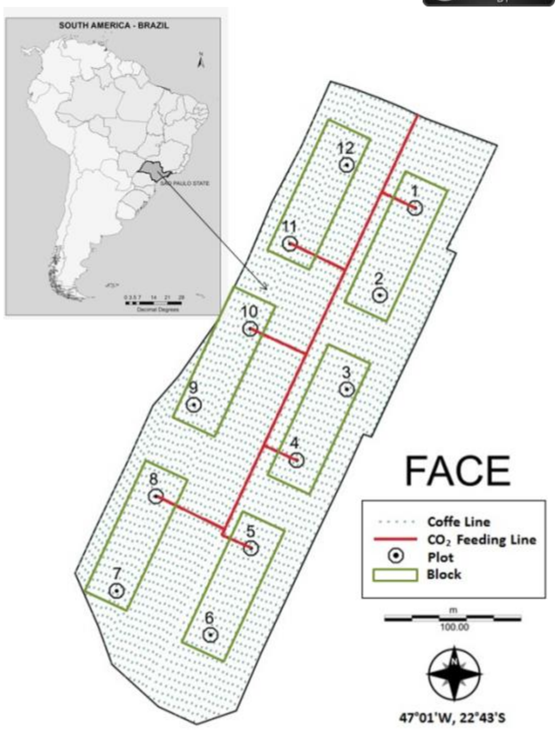

Figure 1. FACE experiment configuration located in the city of Jaguariuna, Sao Paulo state, Brazil.

The radiometric measurements were performed using the LeafClip/Plant Probe attached to the ASD FieldSpec Pro radiometer from 300nm to 2,500nm spectral range, resulting in directional-hemispherical reflectance factors (reflectance) from the adaxial surface of each leaf.

From each leaf spectral characterization campaign, 120 average spectra (10 leaves per plot) were generated, 60 spectra being from elevated CO_2, and 60 from ambient CO_2 plots per year. The averages of these 60 spectra were calculated, which represented specific spectral profiles of each treatment per month for each year.

Seven vegetation indices were considered (Table 1) and calculated from each detached leaf as an individual spectral sample. Spectral vegetal indices minimize variation due to extraneous factors and maximize sensitivity to the variable of interest and, therefore, we can find their application to assess the leaf nitrogen concentration (Daughtry et al., 2000), the impact of soil CO_2 concentration on vegetation (Lakkaraju et al., 2010), among others.

Table 1. Vegetation reflectance indices utilized in this experiment, including their acronyms, formulae and reference.

Vegetation index	Formulation	Reference
Chlorophyll Normalized Difference Index	Chl NDI = $(\rho_{750} - \rho_{705})/(\rho_{750} + \rho_{705})$	Richardson, Duigan & Berlyn (2002)
Normalized Diference Nitrogen Index	NDNI = $(\mathrm{Log}(1/\rho_{1510}) - \mathrm{Log}(1/\rho_{1680}))/(\mathrm{Log}(1/\rho_{1510}) + \mathrm{Log}(1/\rho_{1680}))$	Serrano, Peñuelas & Ustin (2002)
Normalized Difference Vegetation Index	NDVI = $(\rho_{760\text{-}850} - \rho_{630\text{-}685})/(\rho_{760\text{-}850} + \rho_{630\text{-}685})$	Tucker (1979)
Photochemical Reflectance Index	PRI = $(\rho_{531} - \rho_{570})/(\rho_{531} + \rho_{570})$	Gamon, Peñuelas & Field (1992)
Pigment Specific Simple Ration Index for Chlorophyll a	PSSRa = ρ_{800}/ρ_{675}	Blackburn (1998)
Pigment Specific Simple Ration Index for Chlorophyll b	PSSRb = ρ_{800}/ρ_{650}	Blackburn (1998)
Structural Independent Pigment Index	SIPI = $(\rho_{800} - \rho_{445})/(\rho_{800} - \rho_{680})$	Peñuelas, Filella & Gamon (1995)

Note: ρ = reflectance factor

The vegetation indices Chl NDI, PSSRa and PSSRb are related to total chlorophyll, chlorophyll a and chlorophyll b contents, respectively. In herbaceous canopies, these indices were sensitive to stress induced by high soil CO_2 concentration (Lakkaraju et al., 2010). In "chaparral" continuous canopies, NDNI vegetation index was associated with Nitrogen and lignin (Serrano, Peñuelas & Ustin, 2002). NDVI is one of the best-known vegetation indices and it has been related to vegetation biophysical parameters, mainly involving vegetation canopies. However, NDVI presented low correlations to chlorophyll content estimation since it is based on wide spectral bands (Zarco-Tejada et al., 2005). For vineyard leaves, PRI and SIPI were more sensitive to carotene concentrations and to the ratio of total chlorophyll and carotene than to total chlorophyll content (Zarco-Tejada et al., 2005). Due to the dynamic relationship between chlorophyll and carotene concentrations, SIPI has been explored as an indicative of vegetation stress (Peñuelas & Filella, 1998). In detached peanut and wheat leaves, SIPI index was significantly related to the leaf water content (Peñuelas & Inoue, 1999) and in herbaceous canopies, SIPI was also sensitive to stress induced by high soil CO_2 concentration (Lakkaraju et al., 2010).

The statistical approach was based on the vegetation index dynamics in order to evaluate the effect of both CO_2 treatment and time, considering years and months. The generalized least square (GLS) technique was applied, which allows the adjustment of the heteroscedasticity (unequal error variances) and the correlation of within-group errors (Fox & Weisberg, 2011, Pinheiro & Bates, 2000) in order to estimate the unknown parameters in a complete linear model. After adjusting the complete model, we used the stepwise backward model selection, adopting Akaike's information criterion (AIC), which identifies the minimal adequate model. For each step, the parameter with the highest non-significant p-value was dropped. We applied the analysis of variance (ANOVA) to the minimal model, adopting $p \leq 0.05$ as significant level. The statistical approach was performed using the package nlme (Pinheiro, Bates, Debroyu & Sarkar, 2016) of R software (R Core Team, 2016).

3. Results and Discussion

Identifying the coffee phenological phases during the growing season of field campaigns of 2014, in April, June, and August, the crop was in leaf bud maturation phenological phase; and in October and December, coffee was in flowering and grain expansion phase. In 2015, in April and June, coffee plants were in the grain maturation phase, in August in senescence, and in October and December, in vegetative and leaf bud formation.

Coffee growth was evaluated in the same FACE facility and period, and total number of leaves per branch, plant height, and steam diameter were higher under elevated CO_2 concentration (Iost, Ghini, Nechet & Bettiol, 2022).

The monthly average vegetation indices calculated under CO_2 treatments are shown in Figure 2. All the indices had their values changed along the months and, in general, in a different way for each year. The Chl NDI vegetation index (Figure 2a) achieved maximum values in June and August of 2014 and in April, June, and August of 2015; after dropping in October, and followed by an increase in December for both years. Maximum values of Chl NDI were related to minimum reflectance values occurred in June and August at 705nm for both years, since Chl NDI associates a normalized relationship between the beginning of near infrared spectral region (NIR) and the red edge spectral regions (Table 1), and increasing Chl NDI is associated with a rise in the total chlorophyll (Richardson, Duigan & Berlyn, 2002).

NDNI is associated with leaf Nitrogen content. NDNI (Figure 2b) presented differences in the pattern between 2014 and 2015. In 2014, NDNI values were minima in October, corroborating Chl NDI, and, in 2015, its values were more stable. This different behavior between 2014 and 2015 is possibly due to an expressive drought condition experienced in 2014 (Coelho, Cardoso & Firpo 2015), since NDNI considers reflectance factors at short wave infrared (SWIR) region (Table 1) and this spectral region is strongly influenced by the leaf internal moisture (Kumar, 1974). Comparing the treatments, the values of NDNI were lower in elevated CO_2 than those in ambient CO_2 for almost all the months, except for August 2014 and October 2015.

NDVI dynamic is usually associated with leaf biomass. Minimum values of NDVI (Figure 2c) occurred in October of 2014 and 2015, when the coffee leaves achieved their minimum biomass level.

PRI dynamic indicates the potential photochemical activity. In 2014, PRI (Figure 2d) decreased from April to August, followed by an increase until December. Similar pattern occurred in 2015, except in April. Higher values of PRI were observed in June, October, and December of 2015, compared to 2014 in each month; possibly due to a synergic interaction between precipitation levels (higher in 2015) and coffee phenological phases. The average values of PRI were lower under elevated CO_2 than under the ambient CO_2 in all months of 2014 (Figure 2d).

PSSRa and PSSRb are associated with the contents of chlorophyll a and b, respectively (Blackburn, 1998). The curves of PSSRa and PSSRb (Figures 2e and 2f) had, in general, similar pattern since their formulations (Table 1) include similar spectral regions, but values of PSSRb were higher than of PSSRa, indicating a possible

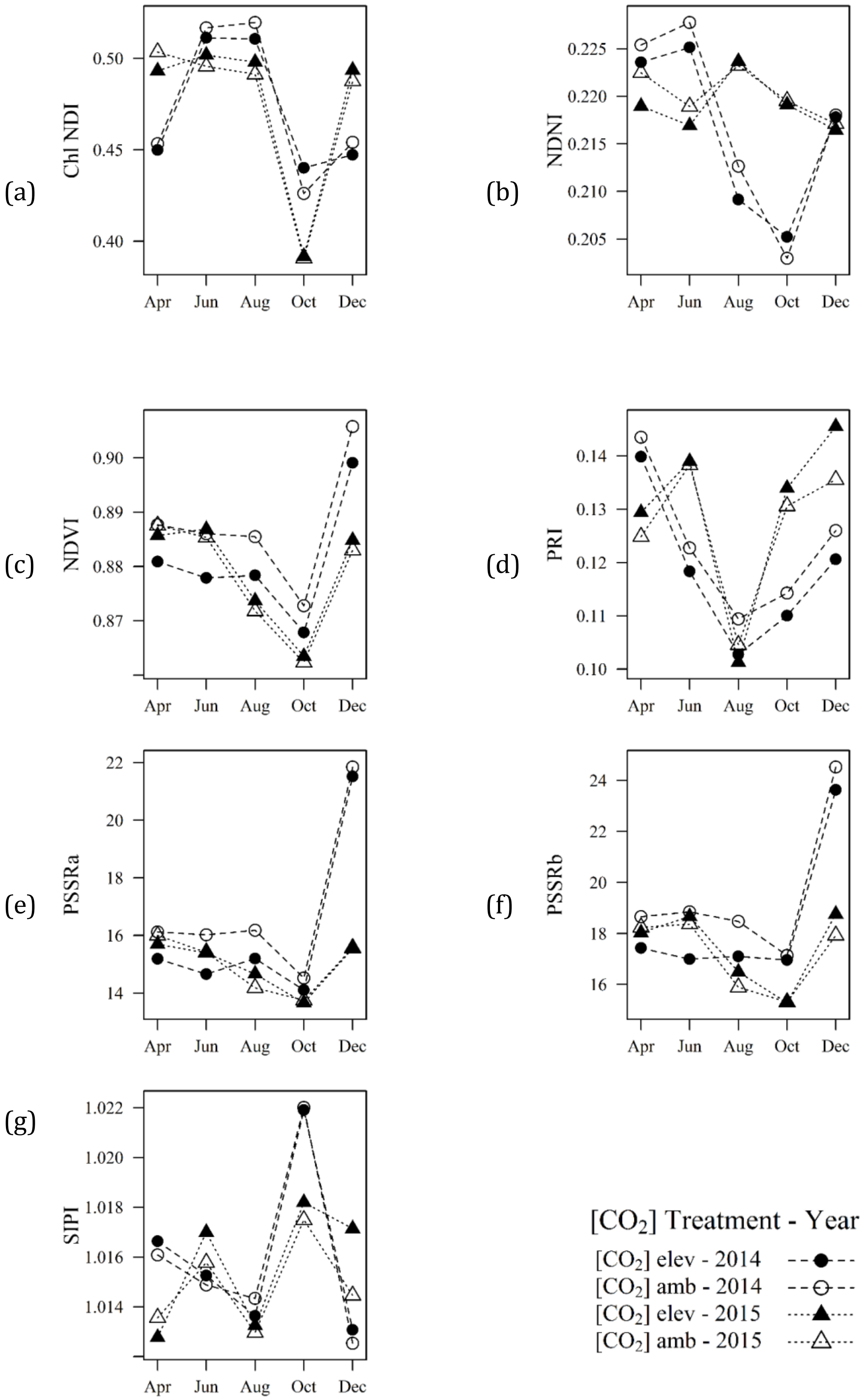

Figure 2. Vegetation indices calculated in 2014 and 2015 under elevated CO_2 (550 µmol mol-1) and ambient CO_2 (390 µmol mol-1). (a) Chl NDI; (b) NDNI; (c) NDVI; (d) PRI; (e) PSSRa; (f) PSSRb; and (g) SIPI.

predominance of chlorophyll b on coffee leaves. RSSRa and PSSRb also followed a similar pattern presented by NDVI. With nearly steady values in the first months (April and June), followed by a decrease in October and a higher increase in December for both years (Figures 2c, 2e, and 2f). In October, they all showed the lowest values, including Chl NDI, reinforced by the lower reflectance values observed at 800nm in NIR spectral region in this month.

Minimum SIPI values (Figure 2g) were observed in August and December of 2014 and in April, August, and December of 2015, indicating that for these months, the leaf internal moisture presented higher levels.

The ANOVA results are presented in Table 2. The two-way interactions between month x year indicate that the changes in the values along the time were statistically significant for all indices. Most vegetation indices, Chl NDI, NDNI, NDVI, PSSRa, and PSSRb showed heteroscedasticity and so, we considered different variances for each year and month in their respective covariance matrix Σ, except for PRI and SIPI, which did not show evidence of heterogeneous variance.

Table 2. ANOVA for the simplified model of each vegetation index. Significant effects ($p \leq 0.05$) are shown in bold.

		Chl NDI[(1)]	NDNI[(1)]	NDVI[(1)]	PSSRa[(1)]	PSSRb[(1)]	PRI	SIPI
Effect[(2)]	numDF	F-stat (p-value)						
Block	5	**8.82 (<0.0001)**	**4.23 (0.0015)**	0.40 (0.8468)	0.57 (0.7251)	1.34 (0.2543)	1.43 (0.2184)	**6.60 (<0.0001)**
CO_2	1	ee	**6.52 (0.0121)**	**8.04 (0.0055)**	**7.48 (0.0073)**	**5.60 (0.0198)**	3.40 (0.0680)	ee
Year	1	**123.14 (<0.0001)**	3.78 (0.0546)	5.02 (0.0272)	3.31 (0.0717)	2.29 (0.1335)	5.37 (0.0255)	**11.30 (0.0011)**
Month	4	**58.10 (<0.0001)**	**36.10 (<0.0001)**	**7.11 (<0.0001)**	**18.75 (<0.0001)**	**11.16 (<0.0001)**	**21.03 (<0.0001)**	**28.30 (<0.0001)**
CO_2 x year	1	ee	ee	**5.42 (0.0218)**	**4.08 (0.0461)**	**5.22 (0.0244)**	**4.48 (0.0366)**	ee
Year x month	4	**33.14 (<0.0001)**	**29.66 (<0.0001)**	**5.11 (0.0008)**	**12.28 (<0.0001)**	**7.89 (<0.0001)**	**13.87 (<0.0001)**	**9.90 (<0.0001)**

[(1)] Index showed heteroscedasticity (year x month).

[(2)] Three-way interaction among CO_2 x year x month and two-way interaction between CO_2 x month excluded in simplification process by AIC.

ee – Effect excluded in model simplification by AIC.

The three-way interaction among CO_2 x year x month and the two-way interaction between CO_2 x month were excluded in the simplification process adopting AIC. The values of Chl NDI and SIPI oscillated along the months and years; however, the effects of CO_2 treatments were not statistically significant (Table 2). That is, the Chl NDI and SIPI were not able to show significant differences between CO_2 treatments. PRI presented CO_2 effect, but it was not in significant way ($p \leq 0.05$).

The two-way interactions between CO_2 x year, for each year, are shown in Table 3 adopting the average test for the vegetation indices, excluding Chl NDI and SIPI. Among the indices considered, NDNI showed significant CO_2 effect and no CO_2 x year interaction. This implies that, regardless of the year, NDNI could identify the CO_2 treatment and the mean value of the index was significantly lower for elevated CO_2 than for ambient CO_2.

Table 3. Averages of vegetation indices[1] considering the CO_2 treatment and the year.

Index[2]	Year	(CO_2) amb	(CO_2) elev
NDNI[3]		0,219a	0,217b
NDVI	2014	0,888Aa	0,881Ab
	2015	0,878Ba	0,879Aa
PRI	2014	0,123Aa	0,118Ba
	2015	0,127Aa	0,130Aa
PSSRa	2014	16,959Aa	16,110Ab
	2015	15,017Ba	14,949Ba
PSSRb	2014	19,513Aa	18,438Ab
	2015	17,217Ba	17,371Ba

[1] SIPI and Chl NDI indices did not show significant difference in CO_2 treatments.

[2] For each index, averages followed by different uppercase in the column and different low case in the row are significantly different from each other (Tukey test, $p \leq 0.05$).

[3] Index did not show the two-wav interaction between CO_2 x vear.

The vegetation index dynamics is related to a complex interaction between climate conditions and crop growing cycle. The significant differences between treatments of CO_2 were detected by the application of vegetation indices from coffee leaves reflectance spectra. The same result was observed for herbaceous vegetation cover submitted to different soil CO_2 content (Lakkaraju et al., 2010). Differences on seasonal vegetation indices performance detected the influence of atmospheric CO_2 on the development of soybean (Gray, Dermody & DeLucia, 2010).

4. Conclusions

In the present study, seven established remote sensing indices were assessed to investigate whether the effect of elevated atmospheric CO_2 could be detected in leaf-level responses of coffee plantation growing under field conditions provided by FACE facility. All the indices exhibited statistically significant changes

in values throughout the two-year growing season, capturing the differences in various coffee development phases. Among these indices, NDNI emerged as a sensitive indicator of the impact of the elevated atmospheric CO_2 on coffee leaves. Additionally, NDVI, PSSRa, and PSSRb demonstrated sensitivity in estimating the effects of elevated CO_2, only under drought conditions. On the other hand, Chl NDI, PRI, and SIPI did not exhibit any sensitivity to elevated atmospheric CO_2. A complex interaction between climate conditions and crop growing cycle alters the dynamics of vegetation indices, making them a prominent tool for assessing the rising CO_2. For future studies, NDNI is a promising candidate for assessing the potential persistency of elevated CO_2 effects from leaf to canopy levels throughout the growing season. Therefore, further studies are needed to assess the applicability of spectral indices and to investigate the effects of biotic and abiotic stresses, including the effects of elevated atmospheric CO_2 on coffee plantations.

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