# Carbon capture, photosynthesis, and leaf gas exchange of shade tree species and Arabica coffee varieties in coffee agroforestry systems in Veracruz state, Mexico (#114940)

Second revision

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# Carbon capture, photosynthesis, and leaf gas exchange of shade tree species and Arabica coffee varieties in coffee agroforestry systems in Veracruz state, Mexico

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Agroforestry systems incorporating native vegetation enhance climate adaptation and mitigation by improving coffee farm resilience, carbon storage, and income diversification. Seven native tree species were previously selected as shade providers for Veracruz coffee agroforestry systems based on ecological, cultural, and economic criteria. The present study evaluated their physiological performance through above-ground biomass, carbon stocks, and in-situ chlorophyll fluorescence and gas exchange measurements under controlled light and temperature conditions. Concurrently, five Coffea arabica varieties were assessed under these shade canopies using the same leaf-level parameters, and leaf nitrogen and moisture content. Erythrina americana and Persea schiedeana showed the highest carbon sequestration per tree. E. americana showed higher water-use efficiency compared to the other, while P. schiedeana showed the lowest transpiration and stomatal conductance, suggesting strong stomatal closure and water loss. Inga inicuil achieved the highest carbon capture per hectare due to its high tree density, despite lower individual tree performance. Physiological analyses identified species-specific strategies. Psidium guajava and P. schiedeana exhibited stomatal regulation, showing high transpiration despite limited carbon gain. E. americana and Inga punctata formed a drought-resilient group, prioritising carbon fixation over water conservation. Intermediate species (Heliocarpus appendiculatus, Inga vera, I. inicuil) balanced moderate CO2 fixation rates with adaptable stomatal response. Photochemical efficiency remained stable across species. Shaded Coffea arabica var. Oro Azteca showed higher leaf nitrogen, moisture content and water-use efficiency, indicating shade-induced improvements in physiological and biochemical parameters linked to growth, photosynthesis, and abiotic stress resilience. PAR levels under shade tree species were lower than in unshaded coffee, suggesting long-term productivity and photoprotection benefits. Principal component analysis showed that PC1 correlated strongly with stomatal conductance and transpiration, driven by P. guajava and P. schiedeana. PC2 showed a carbon economy trade-off between $CO_2$ assimilation and internal concentration, dominated by E. americana. Collectively, these components highlight stomatal regulation and carbon management as adaptive strategies. Coffee PCA contrasted water-use strategies: PC1 showed inverse relationships in stomatal regulation, particularly evident in shaded varieties, while PC2 revealed an energy allocation trade-off between photochemical efficiency and carbon fixation, where shaded plants maintained stable $CO_2$ fixation regarding unshaded ones. These findings provide physiological criteria for selecting shade trees tailored to local climate and production goals, optimising Veracruz agroforestry systems for carbon sequestration, productivity, and sustainability.

# Carbon capture, photosynthesis, and leaf gas exchange of shade tree species and Arabica coffee varieties in coffee agroforestry systems in Veracruz state, Mexico.

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## Abstract

Agroforestry systems incorporating native vegetation enhance climate adaptation and mitigation by improving coffee farm resilience, carbon storage, and income diversification. Seven native tree species were previously selected as shade providers for Veracruz coffee agroforestry systems based on ecological, cultural, and economic criteria. The present study evaluated their physiological performance through above-ground biomass, carbon stocks, and *in-situ* chlorophyll fluorescence and gas exchange measurements under controlled light and temperature conditions. Concurrently, five *Coffea arabica* varieties were assessed under these shade canopies using the same leaf-level parameters, and leaf nitrogen and moisture content. *Erythrina americana* and *Persea schiedeana* showed the highest carbon sequestration per tree. *E. americana* showed higher water-use efficiency compared to the other, while *P. schiedeana* showed the lowest transpiration and stomatal conductance, suggesting strong stomatal closure and water loss. *Inga inicuil* achieved the highest carbon capture per hectare due to its high tree density, despite lower individual tree performance. Physiological analyses identified species-specific strategies. *Psidium guajava* and *P. schiedeana* exhibited stomatal regulation, showing high transpiration despite limited carbon gain. *E. americana* and *Inga punctata* formed a drought-resilient group, prioritising carbon fixation over water conservation. Intermediate species (*Heliocarpus appendiculatus*, *Inga vera*, *I. inicuil*) balanced moderate $CO_2$ fixation rates with adaptable stomatal response. Photochemical efficiency remained stable across species. Shaded *Coffea arabica* var. Oro Azteca showed higher leaf nitrogen, moisture content and water-use efficiency, indicating shade-induced improvements in physiological and biochemical parameters linked to growth, photosynthesis, and abiotic stress resilience. PAR levels under shade tree species were lower than in unshaded coffee, suggesting long-term productivity and photoprotection benefits. Principal component analysis showed that PC1 correlated strongly with stomatal conductance and transpiration, driven by *P. guajava* and *P. schiedeana*. PC2 showed a carbon economy trade-off between $CO_2$ assimilation and internal concentration, dominated by *E. americana*. Collectively, these components highlight stomatal regulation and carbon management as adaptive strategies. Coffee PCA contrasted water-use strategies: PC1 showed inverse relationships in stomatal regulation, particularly evident in shaded varieties, while PC2 revealed an energy allocation trade-off between photochemical efficiency and carbon fixation, where shaded plants maintained stable $CO_2$ fixation regarding unshaded ones. These findings provide physiological criteria for selecting shade trees tailored to local climate and production goals, optimising Veracruz agroforestry systems for carbon sequestration, productivity, and sustainability.

**Subjects:** Biodiversity, Conservation Biology, Natural Resource Management, Environmental Impacts.

**Keywords:** ~~Agroecology; Carbon sequestration; Climate change mitigation; Climate resilience; *Coffea arabica* varieties; Coffee physiology; Ecosystem services; Smallholder coffee farmers; Sustainable agriculture.~~

## Introduction

Coffee is one of the most widely traded and consumed agricultural commodities worldwide (FAO, 2022) with 70% produced in Latin America (Baffes et al., 2005). Coffee production during 2023 was estimated at 10.1 million tonnes, with an expected growth rate of 5.8% by 2024 (FAO, 2022; ICO, 2023). During 2017–2022, 70% of the world's coffee production was exported from producing nations to other countries, generating USD 19 billion in revenue and employing 125 million people globally (Panhuysen and Pierrot, 2020; Fairtrade Foundation, 2022). The coffee industry was valued at US$132.13 billion in 2024, and globally, there are 12.5 million coffee farms, many located in high-biodiversity zones and managed by smallholders (Donald, 2004; Panhuysen and Pierrot, 2020; Mordor Intelligence, 2024). In Mexico, coffee is produced on approximately 580,000 ha by 481,000 farmers (Ellis et al., 2010; Harvey et al., 2021; USDA FAS, 2023). Approximately 86% of production in the country comes from *Coffea arabica* L. cultivation in shade polycultures that support biodiversity and provide ecosystem services (Beer et al., 1998; Moguel and Toledo 1999; Dávalos-Sotelo et al., 2008; Jha et al., 2011; Toledo and Moguel 2012).

Mexican coffee production, which has historically resisted intensification (Rice, 1999), now faces climate-induced land use changes threatening biodiversity (Toledo and Moguel, 2012) and ecosystem services (Beer et al., 1998). Coffee is highly climate-sensitive, with projected range reductions due to shifting temperature, humidity, and rainfall patterns (Bunn et al., 2015; Pham et al., 2019; Bilen et al., 2023). Agroforestry systems mitigate these impacts through shade-regulated microclimates, carbon capture (Verchot et al., 2007; Noponen et al., 2013; Rahn et al., 2014; Jawo et al., 2022; Terasaki Hart et al., 2023), and soil conservation (Segura et al., 2006; Lin, 2007; 2010; Siles et al., 2012; Notaro et al., 2014; Ehrenbergerová et al., 2018), while diversifying income via biomass and other attributes of native trees (Acevedo et al., 1992; Jose, 2009; Häger, 2012; Noponen et al., 2013; Gross et al., 2022). Optimal shade species should be native to enhance soil fertility and pest control without compromising biodiversity (Gill and Prasad, 2000; Reigosa et al., 2000; Gliessman, 2015). The Mexican state of Veracruz exhibits exceptional biodiversity (Rzedowski, 1978; Estrada-Contreras et al., 2015; Tellez et al., 2020), making its coffee agroforestry systems particularly valuable for studying carbon sequestration. These systems produce 23% of Mexico's coffee (Nestel, 1995) and can store 73.27 Mg C ha$^{-1}$ in coffee-tall tree combinations (Ortiz-Ceballos et al., 2020), retaining 91.2% of forest cover while supporting biodiversity comparable to secondary forests (Dávalos-Sotelo et al., 2008; Vizcaíno-Bravo et al., 2020). This evidence highlights their dual role in climate mitigation and conservation.

Plant photosynthesis is pivotal for $CO_2$ mitigation through carbon assimilation into biomass (Fini et al., 2023; Jin et al., 2023). Accurate carbon sequestration estimates rely on above-ground biomass measurements using allometric equations, yielding realistic estimates of assimilated carbon (C) into forest biomass and, consequently, of carbon cycling in ecosystems (Liang and Wang, 2020; Araza et al., 2022). Physiological assessments, particularly leaf gas exchange and chlorophyll fluorescence parameters, offer critical insights into performance and plant acclimatation to environmental changes (Genty et al., 1989; Sakshaug et al., 1998; Roháček, 2002; Baker and Rosenqvist, 2004; Strasser, 2004; Baker, 2008), enhancing the comprehension of ecosystem carbon dynamics.

Shade trees in agroforestry systems critically regulate microclimatic conditions, affecting the physiological responses of coffee plants and associated tree species. Evidence demostrates that shade reduces air temperatures by 1–5 °C, alleviating heat stress and maintaining optimal leaf temperatures (20–24 °C) for photosynthesis (Vaast et al., 2005; Lara-Estrada et al., 2023). These conditions improve chlorophyll fluorescence parameters including Fv/Fm (quantum yield of PSII) by reducing photodamage under excessive irradiance (Rodriguez-López et al., 2014). However, shade-adapted leaves typically exhibit lower heat tolerance than sun leaves, as observed in tropical trees like *Inga spectabilis* (Vahl) Willd., with shade leaves exhibiting a reduced threshold for PSII dysfunction (Slot et al., 2019). Light availability substantially mediates leaf gas exchange in coffee plants. Shade levels of 30–50% optimise photosynthetic rates through balanced irradiance and photoprotection, whereas excessive shade (>60%) can reduce light-saturated photosynthesis and yield by 10–30% (DaMatta, 2004; Haggar et al., 2021; Isaac et al., 2024). Notably, shade-tree traits such as layered canopies or high leaf nitrogen enhance nutrient cycling and light diffusion, further modifying these physiological responses (Sauvadet et al., 2019; Isaac et al., 2024).

Under climate change scenarios, water availability represents a critical challenge for agricultural and natural ecosystems. Water-use efficiency (WUE), defined as the $CO_2/H_2O$ ratio during gas exchange (Kirkham, 2005), couples plant productivity to water management (Bhattacharya, 2019; Hatfield and Dold, 2019). WUE exhibits interspecific variation according to plant functional traits and environmental conditions (Chaves et al., 2004; McCarthy et al., 2011). Agroforestry shade enhances WUE through increased ambient humidity (10–20%) and reducing stomatal conductance, although shaded coffee plants frequently exhibit higher mass-specific transpiration due to morphological adaptations like thinner leaves (Lin 2010; Sarmiento-Soler et al., 2019; de Carvalho et al., 2021; Koutouleas et al., 2022). Collectively, these findings demonstrate shade's role in microclimate stabilisation and photosynthetic optimisation, while highlighting knowledge gaps regarding species-specific physiological trade-offs.

Therefore, the present work examines the physiological characteristics of seven previously selected shade tree species and five *C. arabica* varieties that make up agroforestry systems in central Veracruz, Mexico. For this task, *in-situ* dendrometric measurements of aboveground biomass, chlorophyll fluorescence, gas exchange parameters, and nitrogen and moisture content of leaves located at the understory layer of the selected shade tree species and coffee varieties were conducted under controlled temperature and Photosynthetic Photon Flux Density (PPFD) conditions.

We hypothesised that the physiological traits of these seven tree species identified by Flores-Ortiz et al. (2025) and their interactions with coffee varieties growing in shaded environments enhance carbon capture capacity while improving agroforestry system resilience under climate change. These effects are mediated through stress reduction via shading and optimised resource-use efficiency. We expect that this information will help decision-making during the selection and management of suitable shade trees based on their physiological characteristics and carbon capture potential in shade coffee agroforestry systems.

## Materials and Methods

### Selection of shade tree species and Arabica coffee varieties in agroforestry systems

Seven native tree species were selected from an initial screening of 50 conducted by Flores-Ortiz et al. (2025) based on conservation status, growth rate, and agroecological utility for coffee production. Species used for firewood or fuel were excluded, along with cultivated *Persea americana* Mill. (Hass Avocado) due to its intensive domestication. For the selected shade trees species: *Inga inicuil* Schltdl. and Cham. Ex G. Don (*Ii*); *Inga vera* Willd. (*Iv*); *Inga punctata* Willd. (*Ip*); *Erythrina americana* Mill. (*Ea*); *Psidium guajava* L. (*Pg*); *Persea schiedeana* Turcz. (*Ps*), and *Heliocarpus appendiculatus* Nees (*Ha*), dendrometric, chlorophyll fluorescence, and gas exchange characteristics were measured in trees aged ≤30 years.

Due to the heterogeneity of *C. arabica* varieties found in the coffee farms in the region, chlorophyll fluorescence and gas exchange parameters measured were focused on Oro Azteca (Oa), Garnica (G), Costa Rica 95 (Cr), Tipica (T), and Catuai amarillo (Ca) plants aged 4–6 years. To enable comparison, identical physiological measurements were conducted for Oro Azteca variety plants under unshaded conditions.

### Study area

The shade tree species and coffee varieties studied make up agroforestry systems in a traditional polyculture configuration and in the intermediate secondary succession stage. Coffee plants in unshaded conditions were part of an unshaded monoculture system. The tree density, considering trees between 10–15 m in height and >5 cm in diameter at breast height (DBH, at 1.30 m) for this type of coffee agroforestry system and coffee region, has been reported at ≈1000 trees ha$^{-1}$ (López-Gómez et al., 2008; Williams-Linera and Lorea, 2009).

Study sites were located in central Veracruz on shaded and unshaded coffee farms in the municipalities of Teocelo (19° 23' 36" N, 96° 59' 9.4" W, at an elevation of 1117 m a. s. l., average air temperature of 23.43 ± 0.37 °C and 70.4 ± 5.95% of relative humidity, or RH) and Xico (19° 25' 23.5" N, 96° 55' 42.6" W, at an elevation of 1053 m a. s. l., average air temperature of 27.53 ± 0.86 °C and 60.23 ± 4.16% of RH for shaded and unshaded conditions, respectively (Fig. 1; Map created using the Free and Open Source QGIS).

To ensure environmental homogeneity, measurements were taken during two consecutive cool-season months (October–November 2022). This period exhibited typical climatic conditions for 2016–2022 trends, with precipitation, temperature, and cloud cover within expected ranges (Weather Spark, 2024). The cool season brought average maxima of 24 °C (Teocelo) and 26°C (Xico), alongside ≥1 mm/h precipitation and 80% cloud cover. Historical records show Teocelo's temperature extremes (21–35 °C, 1945–2020), while Xico ranged from 9–12 °C (minima) to 21–22 °C (maxima, 1966–2023) (Servicio Meteorológico

Nacional, www.smn.conagua.gob.mx). Both sites share comparable annual precipitation (1.7 mm) and stable conditions due to their proximity.

**Dendrometric parameters of shade trees and carbon stocks determination**

Measurements were made of the total height and the DBH of three mature and reproductive individuals of each of the seven shade trees. Using these morphometric parameters, the available allometric equations were applied to calculate each tree's AGB (Table 1). The allometric equations used to calculate the AGBs for *Ha* and *Pg* were at species level, at genus level for *Inga* spp. (*Ii*, *Iv* and *Ip*) and for *Erythrin*a sp. (*Ea*), and at tropical forest level for *Ps* (Rojas-García et al., 2015; Ortiz-Ceballos et al., 2020) (Table 1). AGB was converted to biomass carbon stock (CS) by multiplying by 0.47, representing the standard carbon fraction in tree biomass (IPCC, 2006). CS reflects both tree's ability to grow new cells and its carbon storage potential (IPCC, 2021).

Due to variation in tree age, 10-year normalised CS were obtained by a simple proportionality rule for each tree species. This normalisation enables standardised comparison of carbon storage potential while recognising that species-specific traits, such as shade tolerance and wood density, may influence long-term sequestration rates. Finally, we extrapolated hectare-scale carbon storage using the tree density found in the literature (references in Table 2).

**Chlorophyll fluorescence, gas exchange analyses of shade trees and coffee plants**

*In-situ* measurements were performed on leaves from the seven tree species and associated-coffee plants located under the canopy of the seven tree species, which correspond to five varieties of Arabica coffee (Oa, G, Cr, T, and Ca) using a portable infrared gas analyser (IRGA) LI-6400XT (Licor, Lincoln NE, USA) equipped with a fluorometric cell. For shade trees, we assessed three fully developed leaves attached to the first lateral branches at the base of the trunk (branches closest to the understory layer), between 2–4 m from the ground via ladders and climbing equipment. These branches exhibited sympodial growth with a predominantly horizontal or obliquely orientated architecture, characteristic of species such as *Inga* spp. (Troll model), *Ea* (Champagnat model), and *Pg* (Roux model) (Vester, 2002; de Reffye et al., 2008). Coffee measurements were taken from leaves attached to plagiotropic branches at 1.30–2 m height.

Chlorophyll fluorescence analysis was performed in dark-adapted leaf tissues (30 min). Minimum (F0) and maximum fluorescence (Fm) in light-adapted tissues was measured by the saturation pulse method (λ=630 nm, Q >7000 µmol $m^{-2}$ $s^{-1}$, 6s). Based on these signals, variable fluorescence in the dark (Fv=Fm-F0) and quantum efficiency (Fv/Fm) were calculated (Silva et al., 2010; Rakocevic et al., 2022). Fv/Fm is frequently used to estimate the photochemical efficiency of PSII (Niinemets and Kull 2001; Lepeduš et al., 2005; Zavafer and Mancilla, 2021). After that, the gas exchange parameters $CO_2$ fixation rate, stomatal conductance, transpiration, and intercellular $CO_2$ (Ci) were measured in the same leaves attached to the branch. All measurements were conducted between 9:00 h and 11:00 h under controlled conditions, with the

IRGA operated as an open system with a photon flux density of 1000 μmol $m^{-2}$ $s^{-1}$, a leaf temperature of 25 ºC, and an environmental air $CO_2$ concentration of ~420 ppm.

Subsequent gas exchange measurements, it proceeded to estimate incident solar radiation in the understory layer between shaded and unshaded systems. PAR was measured under all tree species' canopies and sun-exposed Oro Azteca coffee plants at midday; this period sees peak solar radiation and thermal stress (Meili et al., 2021; Kohl et al., 2024).

Instantaneous leaf water-use efficiency ($WUE=A/E$) was calculated as the $CO_2$ fixation-transpiration ratio (Hatfield and Dold, 2019) .

### Gravimetric and analytical chemical analysis: moisture and nitrogen content of coffee leaves samples

Following immediately after chlorophyll fluorescence and leaf gas exchange measurements, the same leaves from Oro Azteca coffee plants grown under both shaded and unshaded conditions were excised at the base of the petiole. Collected leaves were stored in sealed plastic containers to prevent moisture loss until further analysis. Subsequently, the samples were processed within the next two days after field sampling. The leaves from each condition were macerated and pooled, then 0.5 g was taken for moisture and nitrogen analysis. Gravimetric methods comparing weight before and after drying determined moisture content based on the following equation:

$$H\ (\%) = \frac{FW - DW}{FW} \times 100 \qquad (2)$$

This parameter refers to the proportion of water present in fresh leaf tissue relative to its total weight, where *H (%)* is the moisture content expressed as a percentage, *FW* is the fresh weight, and *DW* is the dry weight.

We employed the semimicro-Kjeldahl method (Nelson and Sommers, 1980; DOF, Mexican norm NMX-Y-359-SCFI-2019) for digested 50 mg of dried leaf material using a digestion/distillation apparatus (Labconco®) to quantify organic nitrogen content.

### Statistical analysis

All measurements were assayed in triplicate. Collected data from each individual were averaged per tree and per tree species or coffee variety (n=9). Statistical analyses were conducted on the averaged data. Data from both shade trees and coffee plants met assumptions of normality (Shapiro-Wilk, $p > 0.01$), so no transformations were needed.

One-way ANOVA and the *post-hoc* Tukey test were used to identify significant differences ($p < 0.001$). Comparisons were conducted for each parameter between all shade tree species. Coffee variety comparisons were performed between the Oro Azteca variety in shaded conditions vs unshaded conditions. Additionally, comparisons were conducted considering only coffee varieties in shaded conditions. WUE statistical differences between shade trees and between coffee varieties were identified following the same methodology ($p < 0.001$).

Statistical analyses of Oro Azteca moisture and nitrogen content in shaded conditions vs unshaded conditions were performed using a two-tailed t-test (t(4) = 7.6, $p = 0.002$).

To analyse the possible clustering of the seven shade tree species and coffee varieties based on their physiological traits, a PCA was conducted on both datasets comprising all measured parameters (Fv/Fm, $CO_2$ fixation rate, stomatal conductance, transpiration rate, and intercellular $CO_2$ concentration). PCA analysis proceeded by extracting principal components from the correlation matrix of the variables, with the selection criterion being the retention of components that collectively explained at least 75% of the total variance.

GraphPad Prism® version 9.5.1 for macOS (GraphPad Software, San Diego, CA, USA; www.graphpad.com) was used for all statistical analysis (accessed in January 2023).

## Results

### Dendrometric parameters of shade trees and carbon stocks determination

The trees with the highest average AGB and CS values were *Ea* and *Ps*; *Ha* and *Ii* displayed moderate levels of AGB and CS, while *Iv*, *Pg*, and *Ip* had the lowest. CS 10-year normalised was quite similar for the seven species, showing that *Ps* and *Ea* stood out for their highest carbon storage capacities, followed by lower values for the remaining species (Table 1).

Annual CS per hectare calculated according with the reported tree densities was highest for *Ii*, followed by *Ea*, *Ps*, and *Ha*, which also showed relatively high values. In contrast, *Iv*, *Ip*, and *Pg* had the lowest CS values (Table 2). Carbon capture for *Ea*, *Ps*, and *Ha* represents 31.91%, 17.72%, and 10.03% of the total carbon assimilation, respectively, while *Iv*, *Ip,* and *Pg* together represent 6.53%. It is important to note that, according with the carbon stock per hectare calculated through literature-based tree densities, ~40 trees of *Ea*, *Ps,* and *Ha* represent 94.48%, 52.47%, and 29.71%, respectively, of the carbon assimilated by ~200 *Ii* trees, the species with the highest reported tree density and therefore the highest carbon capture.

### Chlorophyll fluorescence, gas exchange and PCA analyses of shade trees and coffee plants

The Fv/Fm values of shade tree species (range 0.74–0.81) and coffee plants (0.73–0.80) showed consistent trends, with no significant differences observed (Table S1). Similarly, no significant differences were found between shaded and unshaded Oa or among other coffee varieties under shaded conditions (Table S2).

$CO_2$ fixation rate differed significantly among species ($F_{(6, 56)} = 118.3$, $p < 0.001$), with the highest rates observed in *Ea*, *Pg*, and *Ha*, followed by *Ip*, and then *Ps*, *Iv*, and *Ii* (Fig. 2b, Table S1). For Oa coffee variety, $CO_2$ fixation rates differed significantly between shaded and unshaded conditions ($F_{(3, 32)} = 54.42$, $p < 0.001$). Among shaded coffee varieties, *Ip*-Oa and *Ha*-Oa exhibited the highest $CO_2$ fixation rates, while other combinations showed lower values ($F_{(6, 56)} = 16.82$, $p < 0.001$) (Figs. 3a and 4a, Table S2).

Transpiration rates showed significant variation among both tree species and coffee varieties. The highest rates were observed in *Pg* and *Ha*, with *Ii* and *Iv* showing intermediate values, and *Ip*, *Ea*, and *Ps* displaying the lowest rates ($F_{(6, 56)} = 65.43$, $p < 0.001$) (Fig. 2b, Table S1). For the Oa coffee variety, unshaded and shaded *Ip*-Oa exhibited the highest transpiration rates, while *Ha*-Oa and *Ps*-Oa showed the lowest values ($F_{(2, 32)} = 6.488$, $p < 0.001$) (Fig. 3b, Table S2).

Among shade-grown coffee varieties, *Ii*-G demonstrated the highest transpiration rates, while *Ea*-T, *Ha*-Oa, and *Ps*-Oa exhibited the lowest ($F_{(6, 56)} = 17.98$, $p < 0.001$) (Fig. 4b, Table S2). Stomatal conductance exhibited similar patterns to transpiration, demonstrating a strong relationship between these parameters. The highest values were recorded in *Pg*, *Iv*, and *Ha*, with intermediate rates in *Ea*, *Ii*, and *Ip*, and the lowest in *Ps*. Significant differences were observed ($F_{(6, 56)} = 19.33$, $p < 0.001$), particularly between the highest and lowest performers (Figure 2c, Table S1). For the Oa cultivar, stomatal conductance varied significantly between unshaded conditions and shaded *Ha*-Oa and *Ps*-Oa ($F_{(3, 32)} = 8.697$, $p < 0.001$) (Fig. 3c, Table S2). Among shaded coffee varieties, *Iv*-Cr and *Ii*-G showed the highest conductance values, while other combinations, including *Pg*-Ca, *Ip*-Oa, and *Ha*-Oa exhibited lower values ($F_{(6, 56)} = 24.02$, $p < 0.001$) (Fig. 4c, Table S2).

Ci values among shade trees exhibited notable variation, reflecting differences in carbon assimilation rates. The highest values were observed in *Iv*, *Ha*, and *Ii*, with intermediate values in *Ps* and *Ip* and lower values in *Pg* and *Ea*, with significant differences between them ($F_{(6, 56)} = 48.22$, $p < 0.001$) (Fig. 2d, Table S1). For the Oa variety, Ci values were influenced by shaded and unshaded conditions, with *Ip*-Oa showing the highest values and *Ps*-Oa the lowest ($F_{(6, 56)} = 48.22$, $p < 0.001$) (Fig. 3d, Table S2). Among shaded coffee varieties, *Ip*-Oa, *Iv*-Cr, and *Ii*-G demonstrated higher Ci values, while *Ea*-T, *Pg*-Ca, *Ha*-Oa, and *Ps*-Oa showed lower values, showing significant differences between them ($F_{(6, 56)} = 11.89$, $p < 0.001$) (Fig. 4d, Table S2).

PAR levels recorded at midday revealed significant differences among radiation reaching the understory layer under tree species' canopy and cultivation conditions. Among shade tree species, *Ea* exhibited the highest average PAR values (92.2 ± 6.66 $\mu mol\ m^{-2}\ s^{-1}$), followed by *Pg* (59.1 ± 9.20), *Iv* (54.0 ± 14.6), *Ha* (45.7 ± 10.99), and *Ii* (40.0 ± 5.95). The lowest values were recorded in *Ip* (27.6 ± 3.34) and *Ps* (23.3 ± 4.06). Significant differences were observed between shade tree species PAR values ($F_{(6, 56)} = 64.15$, $p < 0.001$) (Table S1). For coffee varieties, unshaded Oa showed dramatically higher PAR values (1427 ± 124.0 $\mu mol\ m^{-2}\ s^{-1}$) compared to shaded conditions. Among shaded coffee varieties, *Iv*-Cr (47.1 ± 9.18) and *Pg*-Ca (32.2 ± 5.38) displayed the highest PAR values, while *Ps*-Oa (8.44 ± 1.08) and *Ha*-Oa (14.8 ± 6.64) showed the lowest measurements, with significant differences between conditions ($F_{(6, 56)} = 47.68$, $p < 0.001$) (Table S2).

WUE values showed significant variation among the seven tree species, ranging from 0.83 ± 0.15 $\mu mol\ CO_2/mmol\ H_2O$ in *Ii* to 16.92 ± 11.05 $\mu mol\ CO_2/mmol\ H_2O$ in *Ea* ($F_{(6, 56)} = 5.810$; $p < 0.001$). The highest WUE values were observed for *Ea* and *Ip*, with *Pg*, *Ha*, and *Ps* showing intermediate values. Lower WUE values were observed in *Ii* and *Iv* (Fig. 5a, Table S1). For the Oa coffee variety, WUE values ranged from 4.61 ± 10.06 $\mu mol\ CO_2/mmol\ H_2O$ in unshaded conditions to 21.62 ± 11.27 $\mu mol\ CO_2/mmol\ H_2O$ in the *Ha*-Oa shaded association ($F_{(3, 32)} = 8.538$; $p < 0.001$) (Fig. 5b, Table S2). Among shaded Arabica cultivars, values varied from 7.12 ± 3.78 $\mu mol\ CO_2/mmol\ H_2O$ in *Iv*-Cr to 21.62 ± 11.27 $\mu mol\ CO_2/mmol\ H_2O$ in *Ha*-Oa; however, no statistically significant differences were observed (Fig. 5c, Table S2).

For the PCA analysis for the seven shade tree species, PC1 accounted for 43.57% of the variance (eigenvalue = 2.178), while PC2 contributed 33.43% (eigenvalue = 1.671), cumulatively explaining 77% of the dataset variability. In contrast, the PCA for the coffee varieties (including Oro Azteca) under shaded and unshaded conditions revealed a stronger influence of PC1, which explained 49.04% of the variance (eigenvalue = 2.452), with PC2 adding 29.98% (eigenvalue = 1.499), resulting in a slightly higher cumulative variance (79.02%) (Table S3). In both analyses, variable loadings, species contributions, and correlations were carefully examined to interpret the biological meaning of each component. However, while the shade tree PCA focused on broad physiological patterns across multiple species, the coffee PCA placed particular emphasis on stomatal behaviour, carbon assimilation dynamics, and water-use efficiency, reflecting the distinct ecophysiological responses between shade trees and cultivated coffee varieties.

The PCA revealed important differences between shade trees and coffee varieties. For the seven shade tree species, PC1 was strongly associated with stomatal conductance (loading = 0.882) and transpiration (0.866). *Pg* and *Ps* contributed most to PC1 (42.5% and 37.1%, respectively). In contrast, PC2 highlighted a trade-off between $CO_2$ fixation rate (0.594) and intercellular $CO_2$ concentration (-0.937), with *Ea* dominating this axis (73.9% contribution). The remaining species exhibited intermediate traits (Fig. 6a, Table S3).

Similarly, the PCA for coffee varieties (including shaded and unshaded *Coffea arabica* var. Oro Azteca) (Fig. 6b, Table S4) identified PC1 as a gradient of water-use strategies, but with inverse relationships: stomatal conductance (-0.906), transpiration (-0.897), and intercellular $CO_2$ (-0.884) showed strong negative loadings. The shaded varieties *Ps*-Oa and *Ha*-Oa contributed most to PC1 (22.0% and 5.9%, respectively). Meanwhile, PC2 emphasised a trade-off between photochemical efficiency (Fv/Fm= 0.766) and $CO_2$ fixation rate (-0.884). Unshaded Oa was an outlier along PC2 (39.3% contribution) (Fig. 6b, Table S4).

Correlation analyses for shade trees, stomatal conductance, and transpiration were strongly positively linked (r = 0.825), while $CO_2$ fixation and intercellular $CO_2$ were negatively correlated (r = -0.689) (Table S3). For coffee varieties, stomatal conductance, transpiration, and intercellular $CO_2$ showed strong positive correlations (r = 0.698–0.731), whereas $CO_2$ fixation and Fv/Fm were moderately negatively associated (r = -0.420) (Table S4).

The PCA results enabled clear functional grouping of both shade trees and coffee varieties, though with distinct clustering patterns. For the seven shade tree species, three groups emerged: 1) Stomatal-based response species (*Pg* and *Ps*) dominating PC1 through high stomatal conductance and transpiration; 2) Carbon-conserving *Ea* influencing PC2 via a $CO_2$ fixation vs intercellular $CO_2$ trade-off; and 3) Intermediate species (*Ii*, *Iv*, *Ip*, *Ha*) showing balanced traits. Similarly, coffee varieties formed three clusters: 1) Stomatal-based response varieties, with *Ps*-Oa and *Ha*-Oa exhibiting strong stomatal regulation on PC1; 2) Unshaded Oa displaying high Fv/Fm but low $CO_2$ fixation on PC2; and 3) Intermediate varieties (*Ii*-G, *Iv*-Cr). Key differences included opposite stomatal conductance loadings (positive in shade trees vs negative in coffee) and coffee's light-adaptation trade-offs (absent in shade trees).

### Gravimetric and analytical chemical analysis: moisture and nitrogen content of coffee leaf samples

The shaded Oa samples showed a slightly higher moisture content (55.56%) compared to the unshaded samples (53.97%). Also, shaded Oa samples exhibited a significantly ($p = 0.002$) higher nitrogen content (2.83 ± 0.06%) compared to unshaded samples (2.54 ± 0.02%).

## Discussion

Our findings highlight the ecological value of seven native tree species in carbon capture, water-use efficiency, and climatic resilience within coffee agroforestry systems in Veracruz, Mexico. Our results demonstrate that the studied tree species contribute differently to the carbon capture of the agroforestry system. The combined physiological characteristics of the tree species and coffee varieties, as well as higher nitrogen and moisture content in shaded coffee plants compared to unshaded ones, support our hypothesis. Overall, the findings highlight the diverse nature of agroforestry systems and the improved microclimatic conditions provided by tree shade.

### Dendrometric parameters of shade trees and carbon stock determination

The seven shade tree species showed distinct photosynthetic traits, growth and biomass allocation patterns, leading to inherent variability in carbon accumulation, making direct interspecific comparisons of AGB and CS unreliable (Poorter et al., 2008; Chave et al., 2014). Works such as that of Garza-Lau et al. (2020) show these types of variations in agroforestry systems in the state of Veracruz. Farmers typically manage tree density through greenhouse propagation or native seedling programmes, though optimal densities require further study to balance competition and productivity. Regional factors, including altitude, which lowers temperatures and affects phenology (Bewley and Black, 1994), slope, and shade management, significantly influence tree traits and ecosystem services (Cerda et al., 2017; Asanok et al., 2024). These environmental constraints, alongside cultivation practices, collectively determine population densities. Regarding the contribution of coffee trees to carbon sequestration in the study area, it is important to note that, although it was not determined in this work, their contribution is significantly variable, which can range from 0 to 12 Mg ha-1 (Valdés-Velarde et al., 2022), which depends on the different varieties and density of coffee plants per hectare, making extrapolation and comparison difficult, even within the same study area.

Variation in carbon storage among the species revealed key functional roles in agroforestry systems. *E. americana* and *P. schiedeana* emerged as the most efficient carbon accumulators at the individual tree level, while *I. inicuil* showed higher performance at the hectare level due to its high population density. This contrast highlights how growth strategies and management decisions collectively shape carbon sequestration potential.

Three ecological patterns emerge when examining species contributions. First, the *Inga* genus (Fabaceae), particularly *I. inicuil*, achieves remarkable carbon capture through numerical dominance rather than individual tree performance. With 660 trees ha$^{-1}$, *I. inicuil* accounts for

33.77% of system carbon uptake, achieving 198 Mg C ha$^{-1}$, nearly double the sequestration reported for other *Inga*, *Erythrin*a, and Musaceae species (91.64–115.5 Mg C ha$^{-1}$; Haber, 2001). Reported densities for *I. inicuil* range from 100 to 800 trees ha$^{-1}$, averaging 250–350 trees ha$^{-1}$ (Barradas and Fanjul, 1986; Soto-Pinto et al., 2001). After 10 years of growth, *I. inicuil* exhibited a CS of 20.9 Mg C ha$^{-1}$ at 200 trees ha$^{-1}$, exceeding values for *Inga densiflora* Benth. (24.3 Mg C ha$^{-1}$ at 400 trees ha$^{-1}$) with similar age and size parameters (Salazar, 1985; Kursten and Burschel, 1993). However, these values were three times lower than those reported for *I. inicuil* in Oaxaca, Mexico (64.3 Mg C ha$^{-1}$ at 164 trees ha$^{-1}$; Hernández-Vásquez et al., 2012; Alessandrini et al., 2011; Téllez et al., 2020), underscoring how regional factors like altitude and microclimate interact with species physiology. Although *I. punctata* had lower AGB and CS, its high density (representing 20–40% of total trees) contributed significantly to system-level carbon capture (Soto-Pinto et al., 2001). Incorporating both *I. punctata* and *I. vera* in agroforestry configurations may achieve carbon stocks of 91.64 Mg C ha$^{-1}$ (Haber, 2001).

Second, *E. americana* and *P. schiedeana* follow a quality-over-quantity approach. Their substantial trunk diameters and heights enable just 40 trees to capture carbon equivalent to 94.48% and 52.47%, respectively of the carbon captured by 200 *I. inicuil* trees. However, biological constraints, including seed dormancy in *E. americana* (Bewley and Black, 1994; Bonfil-Sanders et al., 2008) and extensive crown-canopy development in *P. schiedeana* (Niembro, 1992; Vázquez-Torres et al., 2017), naturally restrict their planting densities in managed agroforestry systems.

Third, the complementary roles of remaining species enhance system functionality. *H. appendiculatus*, representing 16–20% of tree strata in Chiapas coffee farms (Soto-Pinto et al., 2001; Castillo-Capitán et al., 2014), accounted for 10.03% of total carbon assimilation despite representing only 6% of trees in this study. *P. guajava*, which constituted 4–5% of tree density in coffee plantations (Soto-Pinto et al., 2001), showed the lowest CS values (6.53% of total CS at 10 years), consistent with prior findings (Nava et al., 2009) and attributable to its average height of 3–8 meters (Hauzé et al., 2017). *P. guajava* can be incorporated into agroforestry systems to enhance carbon storage, particularly in leaves and roots, with a whole calculated CS ranging between 0.27 and 4.19 Mg ha$^{-1}$ in 2- to 10-year-old orchards (Naik et al., 2021). Additionally, it provides valuable firewood and fruits for human and animal use (Somarriba, 1988; Pascarella et al., 2000; Miceli-Méndez et al., 2008).

Smallholders could optimise carbon capture by combining high-density *Inga* plantings with strategic inclusions of high-efficiency species like *E. americana* and *P. schiedeana*. Such configurations could simultaneously qualify for carbon offset schemes (Magnago et al., 2015; Lal et al., 2015; Zomer et al., 2017) while overcoming adoption barriers like limited land availability (Feliciano et al., 2014; Buck and Palumbo-Compton, 2022).

## Chlorophyll fluorescence and gas exchange analyses of shade trees and coffee plants

Physiological performance among shade trees and coffee varieties revealed distinct functional strategies shaped by interspecific variation in quantum efficiency, stomatal behaviour, and carbon capture potential.

Most species maintained Fv/Fm values above the 0.75 threshold for fully functional PSII (Lepeduš et al., 2005), indicating robust photochemical activity. *E. americana* proved an exception, with lower values potentially linked to reduced intercellular $CO_2$ (Ci), as atmospheric $CO_2$ concentrations influence quantum yield (Genty et al., 1989). This divergence underscores how intrinsic physiological traits interact with environmental conditions to determine carbon capture efficiency.

Principal Component Analysis (PCA) identified three clusters among shade tree species. First, *P. guajava* and *P. schiedeana* exhibited high stomatal conductance and transpiration, suggesting superior photosynthetic efficiency at the cost of greater water loss, a strategy adapted to moisture-replete environments. Second, *E. americana* emerged as an outlier along PC2, with the highest PC score. The observed inverse relationship between $CO_2$ fixation rate and Ci through this PC suggest a water-conserving adaptations prioritising carbon assimilation over internal $CO_2$ accumulation. Third, *I. vera*, *I. punctata*, and *H. appendiculatus* occupied intermediate positions, demonstrating balanced physiological traits without extreme specialisation. The strong negative correlation between $CO_2$ fixation and Ci in PC2 highlights a fundamental trade-off in photosynthetic strategies.

1) Stomatal-regulating shade tree species: *P. guajava* and *P. schiedeana*.

*P. guajava* and *P. schiedeana* showed the highest contributions to PC1, reflecting their higher stomatal performance, as evidenced by their strong loadings for stomatal conductance and transpiration. These high values suggest not only increased stomatal activity and water loss but also potentially enhanced photosynthetic efficiency, indicating optimal water-use efficiency in these species.

*P. guajava* (Myrtaceae) exhibited marked diurnal gas exchange patterns, with peak $CO_2$ assimilation (16–18 $\mu mol\ m^{-2}\ s^{-1}$) occurring at midday (11:00–14:00 h), followed by an evening decline (Nava et al., 2009). This rhythm reflects efficient light utilisation during peak irradiance, though stomatal conductance plummeted by dusk, limiting late-day carbon fixation, a trait shared with other midday-optimising species (Schussler and Westgate, 1988; Romero et al., 2004). Shaded conditions enhanced *P. guajava* assimilation (15.47 vs 11.96 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ in unshaded settings; Idris et al., 2019), aligning with observed stomatal density increases under high light (Shirke and Pathre, 2003; Casson and Gray, 2008). However, its vulnerability to drought and reduced $CO_2$ uptake under stress (Maxwell and Johnson, 2000; Simonin et al., 2012) suggests limited climate adaptability.

Contrastingly, *P. schiedeana* (Lauraceae) showed consistently low stomatal conductance and $CO_2$ fixation under moderate temperatures (23.43 °C) and high relative humidity (70.4%), a response related to metabolic saturation (Azcón-Bieto et al., 2008). Similar to *P. americana* (Useche-Carrillo et al., 2022), its limited assimilation coincided with elevated Ci, indicating saturation kinetics where stomatal closure halts photosynthesis despite available $CO_2$ (Sánchez-

Díaz and Aguirreolea, 2008; Fricker and Willmer, 2012). This phenomenon, observed in other *Persea* species, reflects non-stomatal limitations, possibly due to mesophyll conductance constraints (Flexas et al., 2012). Elevated vapour pressure deficit (VPD) exacerbated this behaviour, reducing conductance and $CO_2$ uptake (Schultze, 1986), shared behaviour with *P. americana* (Bower et al., 1978). While *P. schiedeana* low carbon gain limits competitiveness under high VPD, its efficiency in stable, humid microclimates supports its agroforestry roles. Key divergences emerge in their climate adaptations: *P. guajava* diurnal efficiency suits controlled-light systems, whereas *P. schiedeana* saturation-prone physiology demands stable humidity. Their contrasting water-use strategies, light-responsive stomata in *P. guajava* (Idris et al., 2019) versus humidity-dependent conductance in *P. schiedeana* highlight genus-specific trade-offs between productivity and resilience.

2) Carbon-conserving shade tree species: *E. Americana*.

Dominating PC2 (73.9% contribution), *E. Americana* exemplified a carbon-conserving strategy, with high $CO_2$ fixation rates despite suboptimal Fv/Fm values (<0.75) and lower stomatal conductance. Such traits align with observations in other *Erythrina* species, where light-saturated photosynthesis couples with high water-use efficiency (Nygren, 1995; Davis and Hidayati, 2019). This behaviour reflects adaptation to low-VPD conditions, where elevated leaf water potential enhances stomatal efficiency (Running, 1976; Dai, 2013; Grossiord et al., 2020). This adaptive mechanism enhanced leaf water potential and assimilation efficiency, contrasting with *Inga* species, which maintained slower stomatal closure under similar conditions (Engineer et al., 2016; Xu et al., 2016). Its pioneer ecology and optimal temperature range (~28°C; García-Mateos et al., 2001; Palma-Garcia and Gonzales-Rebeles Islas, 2018) further support their climate resilience. WUE analysis revealed reduced transpiration under high temperature and radiation conditions, prioritising water conservation, a trait advantageous for drought-prone agroforestry.

3) Intermediate Species: *I. inicuil*, *I vera*, *I. punctata*, and *H. appendiculatus*.

This group displayed balanced physiological traits across both principal components, with moderate contributions and no extreme patterns in any single variable. While this suggests a consistent physiological strategy, likely reflecting adaptability to varying conditions, notable interspecific variation was still evident within the group.

*I. inicuil*, *I vera*, *I. punctata* exhibited lower photosynthetic rates ($1.67 \pm 0.38$ to $5.02 \pm 1.34$ $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) compared to other *Inga* species under controlled conditions (10.60–11.65 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$; dos Santos Pereira et al., 2019), likely due to differences in measurement conditions, such as mature leaves vs younger leaves, light intensity, and temperature. All three species reduced stomatal opening under moderate temperatures (23.43°C) and high humidity (70.4%), limiting water loss more than carbon fixation (Shimshi and Ephrat, 1975). Under elevated temperatures, their slower stomatal closure allowed sustained transpiration (Engineer et al., 2016; Xu et al., 2016), albeit at the cost of reduced WUE.

Contrastingly *H. appendiculatus* (Malvaceae), a pioneer species, showed higher $CO_2$ assimilation (3.7–11.6 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) across light regimes (Tinoco-Ojanguren and Pearcy, 1995), with stomatal conductance and Ci positively correlated with carbon gain (Farquhar and Sharkey, 1982). Its plasticity stems from leaf-level adjustments: increased stomatal density under dim light and thicker leaves in high radiation (Fetcher et al., 1983; Friend, 1984), enabling efficiency in both shaded understories and canopy gaps (Stegemann et al., 1996). Unlike *Inga* species, its photosynthesis was unaffected by light quality (Tinoco-Ojanguren and Pearcy, 1995), suggesting broader niche tolerance.

Under high VPD, *Inga* species conserved water via stomatal closure (Sinclair, 2017), while *H. appendiculatus* prioritised carbon fixation, a critical trait for early succession. For agroforestry, this implies *Inga* species stabilise systems under drought, whereas *H. appendiculatus* optimises productivity in variable light.

PAR beneath canopies differed significantly: *E. Americana* and *Inga* species allowed higher understory PAR (365–379 µmol photons $m^{-2}$ $s^{-1}$) due to open canopy structures (dos Santos Pereira et al., 2019), while *P. schiedeana* and *P. guajava* showed marked irradiance attenuation (40–60% reduction) due to their dense foliage (Siles et al., 2012; Idris et al., 2019). *H. appendiculatus* filtered ≈60% of full sunlight, aligning with its gap-adapted ecology (Tinoco-Ojanguren and Pearcy, 1995; Stegemann et al., 1996). Coffee plants beneath *P. schiedeana* and *P. guajava* experienced PAR levels 30–169 times lower than unshaded conditions, mitigating photoinhibition but potentially limiting $C_3$ photosynthesis at extremes (Roháček, 2002; Nava et al., 2009). These PAR disparities highlight trade-offs between photoprotection and light availability for understory crops (Gholipoor et al., 2010; Sinclair, 2017).

*I. punctata* and *E. americana* exhibited the highest WUE, suggesting drought resilience (Beer et al., 1997; Chaves et al., 2004; Sinclair, 2017). While comparative WUE data for these species were scarce, their values surpassed those of urban non-native trees (McCarthy et al., 2011), except for *I. inicuil* and *I. vera*, which showed the lowest WUE. In subtropical forests, WUE increased with altitude due to light exposure and water deficit (Huang et al., 2023), highlighting habitat influences on water-use strategies.

Arabica coffee varieties clustered into three physiological groups: (1) *P. schiedeana*-Oro Azteca and *H. appendiculatus*-Oro Azteca, with high stomatal regulation and WUE but reduced Ci; (2) unshaded *C. arabica* var. Oro Azteca, exhibiting light-adapted but carbon-limited photosynthesis; and (3) intermediate varieties: *I. inicuil*-Garnica and *I. vera*-Costa Rica 95, with balanced traits. These groupings are further supported by the correlation matrix, which reveals strong linkages between stomatal conductance, transpiration and intercellular $CO_2$, while highlighting the competitive relationship between Fv/Fm and $CO_2$ fixation.

Unshaded coffee plants showed low $CO_2$ fixation due to high-radiation, which impedes electron transport and increases photorespiration above 25 °C (Farquhar and Sharkey, 1982; Mosquera-Sanchez et al., 1999). Shaded plants maintained stable $CO_2$ fixation, highlighting the need for microclimate moderation to optimise productivity. Variability in $CO_2$ fixation rates between varieties also suggests differential sugar and starch accumulation during photosynthesis (Riaño et al., 1993; Mosquera-Sanchez et al., 1999).

Unshaded coffee plants showed detrimental effects of temperature above 25 °C on stomatal conductance and $CO_2$ assimilation, since these plants are exposed to higher radiation and VPD, causing the loss guard cell turgor and stomatal resistance (Makino et al., 1994; Nutman et al., 1937; Riaño 1993; Larcher, 1994; Roháček, 2002). On the contrary, shaded plants exhibited more stable $CO_2$ fixation rates, possibly linked to the moderated microclimatic conditions provided by shade tree canopy.

WUE in 14 *C. arabica* cultivars under drought conditions ranged from 4 to 12.5 µmol $CO_2$/mmol $H_2O$ (Reis et al., 2022). Remarkably, our observed values in the shaded *H. appendiculatus*-Oro Azteca variety achieved 21.62 µmol $CO_2$/mmol $H_2O$, double that of drought-tolerant genotypes observed by Reis et al. This enhanced performance correlates with key adaptations like thicker cuticles, fewer xylem vessels, and higher stomatal density (Baliza et al., 2012; Wolfe et al., 2016). Comparative analysis of 21 genotypes revealed generally lower seasonal WUE stability (1.2–3.4 µmol $CO_2$/mmol $H_2O$; Tezara et al., 2022), with only unshaded Oro Azteca plants approaching our observed values.

WUE reflects plant resource optimisation but is limited by short-term measurement scales (Medrano et al., 2007). For agroforestry, selecting high-WUE native trees like *I. punctata* and *E. americana* can enhance sustainability, productivity, and climate resilience (Hatfield and Dold, 2019), aligning ecological and agricultural goals in water-scarce regions.

### Gravimetric and analytical chemical analysis: moisture and nitrogen content of coffee leaf samples

The lower air temperature and light intensity in shaded areas contribute to higher moisture content in coffee leaves by increasing relative humidity, which lowers the VPD (Schwerbrock and Leuschner, 2017). In contrast, coffee plants in unshaded conditions are subjected to environmental variables more likely to trigger plant stress responses compared to shaded plants and previous evidence on the nitrogen content of leaves has demonstrated that, in stressful situations, increased leaf nitrogen availability promotes the activation and maintenance of photoprotective systems to avert photooxidation (Fahl et al., 1994; Ramalho et al., 2000), which allows shaded leaves to adapt more efficiently to different irradiation conditions than fully sun-exposed leaves (Araujo et al., 2008).

Finally, some considerations need to be made regarding our study; such limitations include the restricted number of farms, sampled leaves assessed, as well as the measurement conditions. Future work should involve longitudinal studies across seasons, testing responses to light, temperature, and $CO_2$ under shaded/unshaded conditions. Establishing permanent sample plots and robust experimental designs would minimise biases in individual measurements; however, these approaches are expensive and time-consuming. As part of this, our analysis contributes to the current physiological status description of the tree species and coffee varieties that make up farms in the coffee-growing region.

## Conclusions

This study highlights physiological differences among seven native shade tree species in Veracruz agroforestry systems, shaping their potential roles in carbon sequestration, microclimate regulation, and coffee productivity. *E. americana* showed superior individual carbon storage capacity, combining high WUE with $CO_2$ fixation. In contrast, *I. inicuil* showed greater hectare-level carbon capture due to its high density, exemplifying the trade-off between individual tree efficiency and population-level impact. These distinctions highlight the need for strategic species selection, prioritising *I. inicuil* for rapid carbon storage and *E. americana* and *P. schiedeana* for long-term resilience.

Functional clustering revealed complementary adaptations: *P. guajava* and *P. schiedeana* optimised photosynthesis in moist environments, whereas *E. americana* and *I. punctata* conserved water while maintaining carbon uptake. Intermediate species like *H. appendiculatus* and *I. vera* balanced moderate productivity with environmental flexibility. Such diversity allows tailored agroforestry designs, for instance, pairing light-filtering *P. schiedeana* with drought-tolerant *E. americana* to buffer climate extremes.

Regarding coffee varieties, the Oro Azteca variety under *P. schiedeana* and H. *appendiculatus* shade canopies exhibited strong stomatal regulation and high WUE, though with reduced intercellular $CO_2$, making it suitable for drought-prone areas. Notably, Oro Azteca plants under *H. appendiculatus* canopy achieved exceptional WUE, which needs to be further investigated. In unshaded conditions, the Oro Azteca variety showed high photochemical efficiency but low $CO_2$ fixation, indicating temperature, humidity and light adaptation at the cost of carbon assimilation. Intermediate varieties like Garnica and Costa Rica 95 displayed balanced traits, adapting well to varying shade and water availability. This evidence emphasises the need for variety-specific selection in agroforestry.

The full potential of tropical forests and agroforestry systems, including their capacity to store carbon, requires applied research on tree species selection, propagation techniques, appropriate population densities, and planting configurations. By reducing environmental toxicity and diversifying unstable monoculture economies, these efforts will improve the protection of watersheds, biodiversity, and the environment. Thus, it is essential to establish clear guidelines for the *ad hoc* selection and management of appropriate shade tree species for the various agroforestry systems to maximise the benefits of tree-crop relationships while avoiding the drawbacks.

This work aims to demonstrate that the tree species selection criteria for agroforestry systems based on the different crop types must integrate the available data on the C capture potential and the physiological behaviour of the species to improve an accurate selection process. The evidence provided also increases the knowledge on the capacity of agroforestry systems to reduce stress and create optimal conditions for growth, as well as a better capacity to adapt to climate change through the provision of shade and its related benefits. As a perspective of this work, more fieldwork is required to determine the constraints, the belowground dynamics and interactions that take place in the functionality of agroecosystems at the level of populations of soil microflora and microfauna and plant communities.

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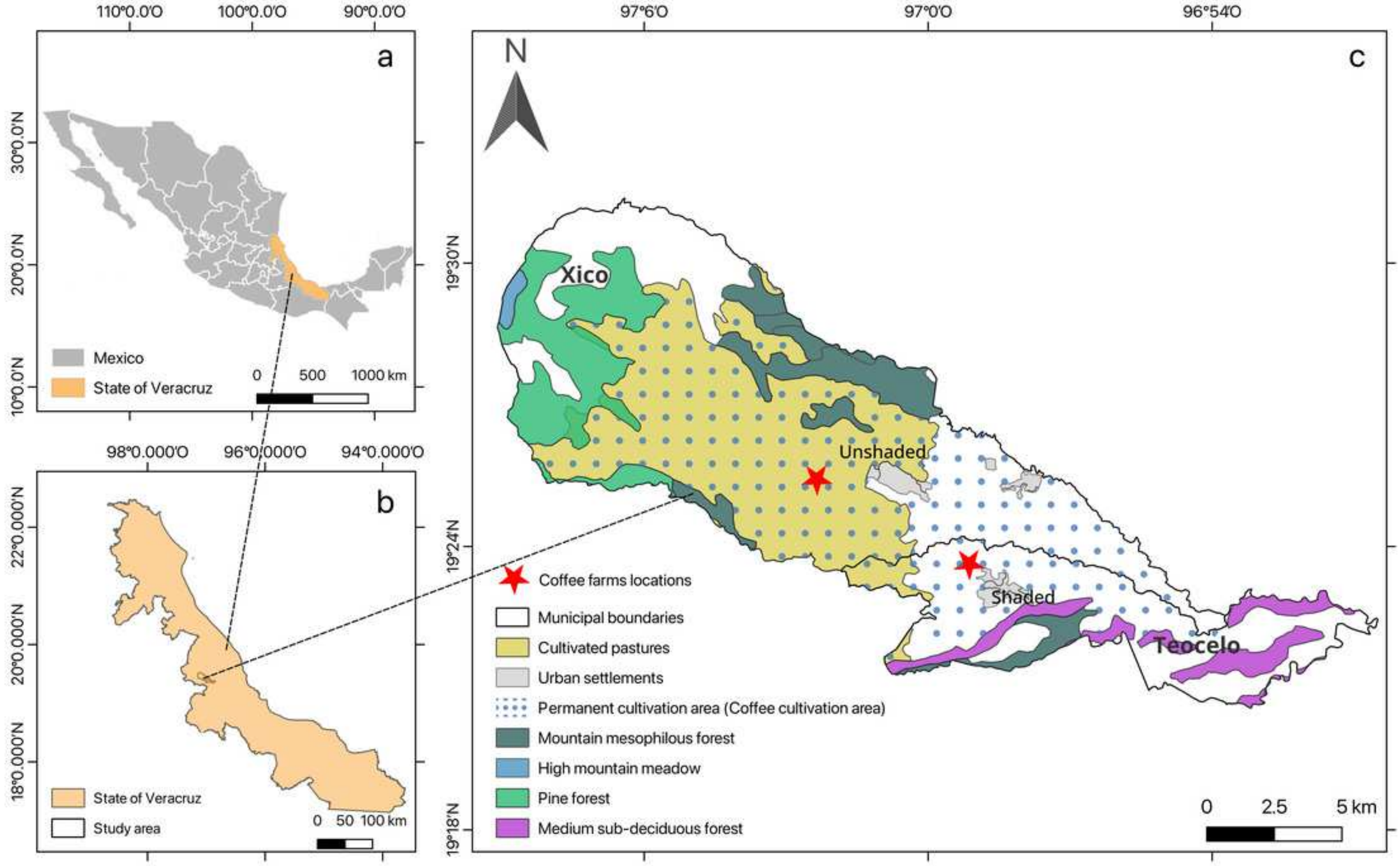
# Figure 1

The study area location.

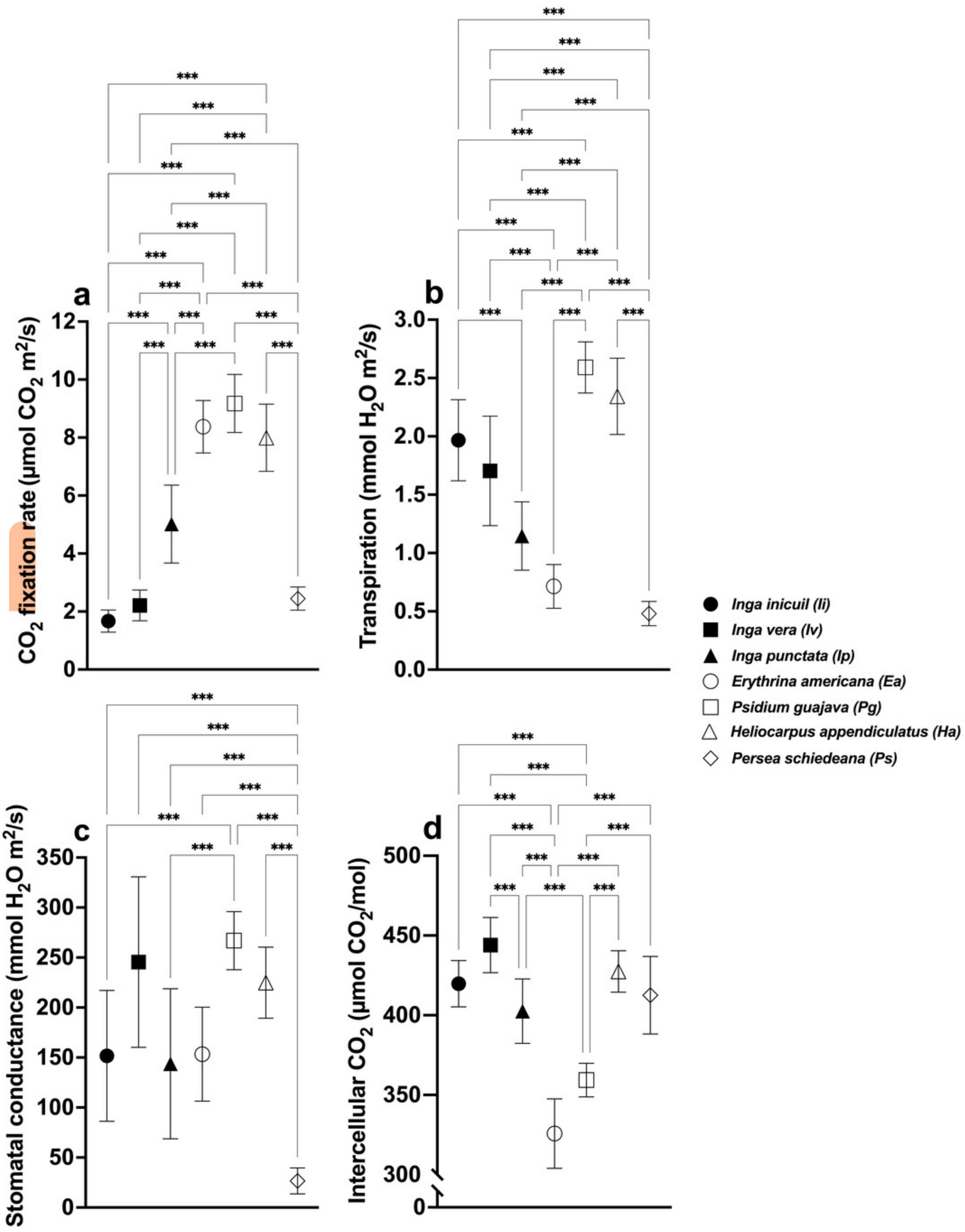
(a) Mexico; (b) the state of Veracruz; and (c) the municipalities with the coffee farms where the *in-situ* tests were conducted.



# Figure 2

Stomatal parameters of shade trees.

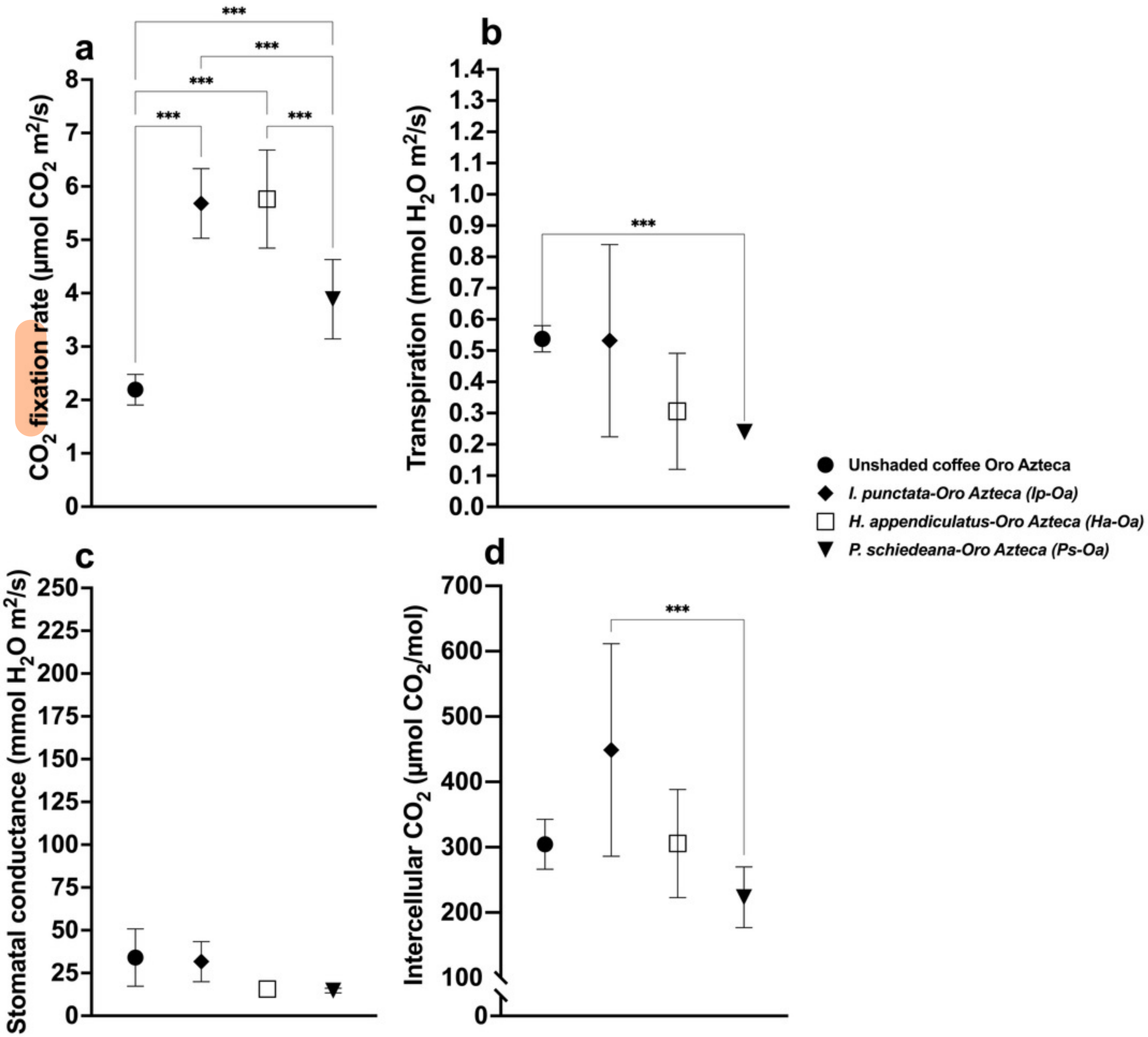
Mean ± SD (n=9). One-way ANOVA and a *post-hoc* Tukey's test were used to identify significant differences. Comparisons with a *p* values ≤ 0.001 (***) are depicted.


a
CO2 fixation rate (µmol CO2 m2/s)
12
10
8
6
4
2
0
b
Transpiration (mmol H2O m2/s)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
c
Stomatal conductance (mmol H2O m2/s)
350
300
250
200
150
100
50
0
d
Intercellular CO2 (µmol CO2/mol)
500
450
400
350
300
0
***
● Inga inicuil (Ii)
■ Inga vera (Iv)
▲ Inga punctata (Ip)
○ Erythrina americana (Ea)
□ Psidium guajava (Pg)
△ Heliocarpus appendiculatus (Ha)
◇ Persea schiedeana (Ps)

# Figure 3

Stomatal parameters of unshaded and shaded coffee plants of the Oro Azteca variety.

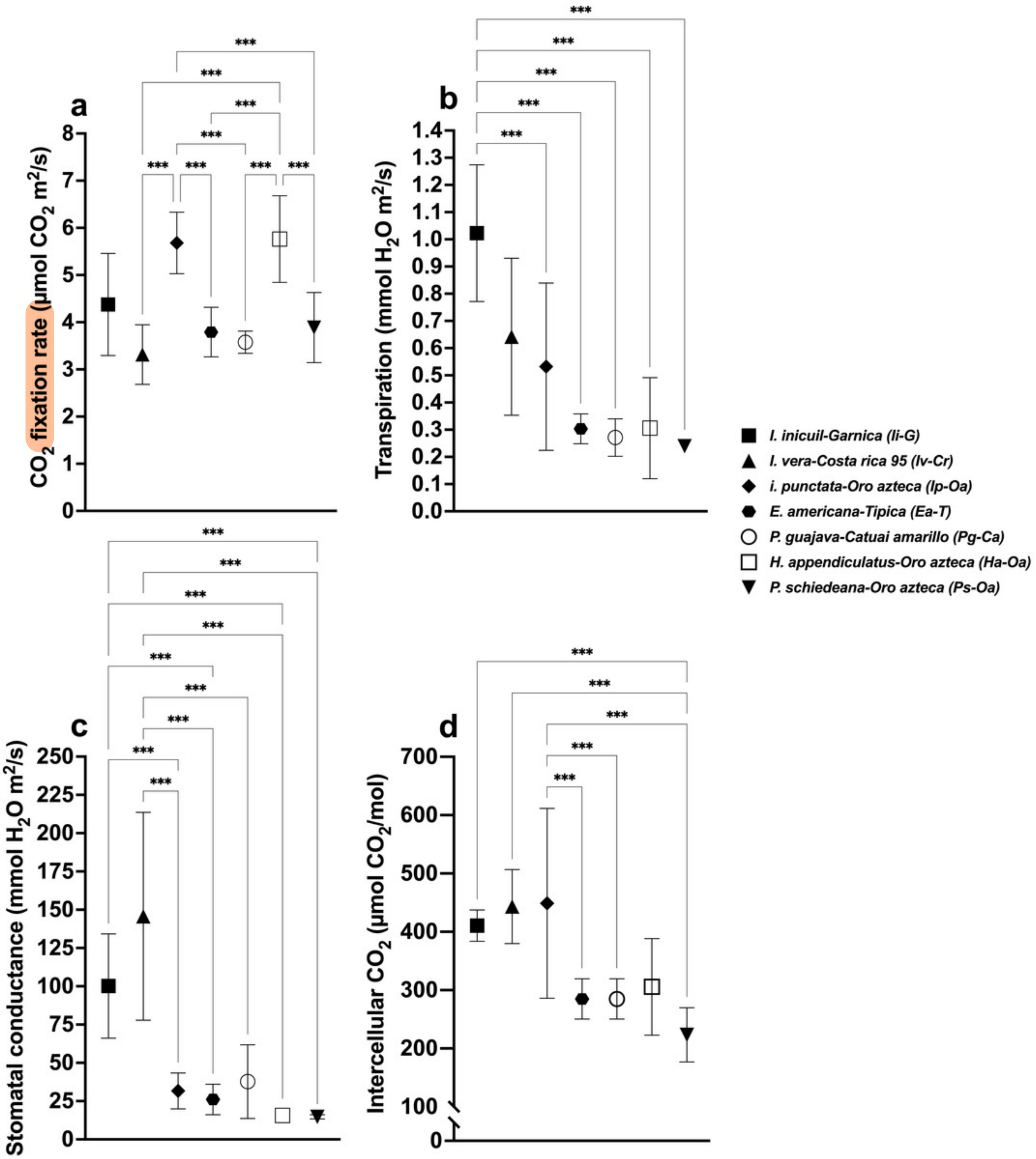
Shade tree species-coffee associations are indicated in each case. Mean ± SD (n=9). One-way ANOVA and a *post-hoc* Tukey's test were used to identify significant differences. Comparisons with a *p* value ≤ 0.001 (***) are depicted.



# Figure 4

Stomatal parameters of the different *Coffea arabica* varieties in shaded condition.

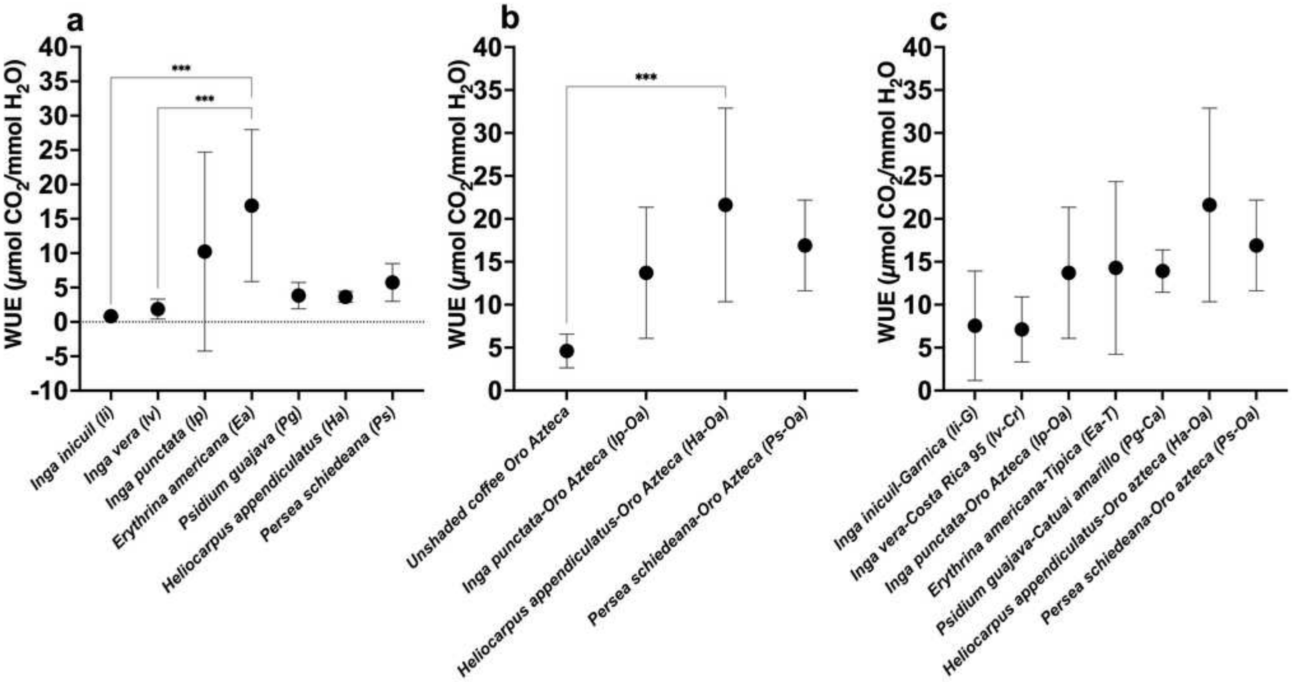
Shade tree species-coffee variety associations are indicated in each case. Mean ± SD (n=9). One-way ANOVA and a *post-hoc* Tukey's test were used to identify significant differences. Comparisons with a $p$ value ≤ 0.001 (***) are depicted.


a
CO$_2$ fixation rate (μmol CO$_2$ m$^2$/s)
0
1
2
3
4
5
6
7
8
***
b
Transpiration (mmol H$_2$O m$^2$/s)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
***
■ I. inicuil-Garnica (Ii-G)
▲ I. vera-Costa rica 95 (Iv-Cr)
◆ i. punctata-Oro azteca (Ip-Oa)
⬢ E. americana-Tipica (Ea-T)
○ P. guajava-Catuai amarillo (Pg-Ca)
□ H. appendiculatus-Oro azteca (Ha-Oa)
▼ P. schiedeana-Oro azteca (Ps-Oa)
c
Stomatal conductance (mmol H$_2$O m$^2$/s)
0
25
50
75
100
125
150
175
200
225
250
***
d
Intercellular CO$_2$ (μmol CO$_2$/mol)
0
100
200
300
400
500
600
700
***

# Figure 5

Instantaneous leaf water-use efficiency (WUE) of shade trees and coffee varieties in unshaded and shaded conditions.

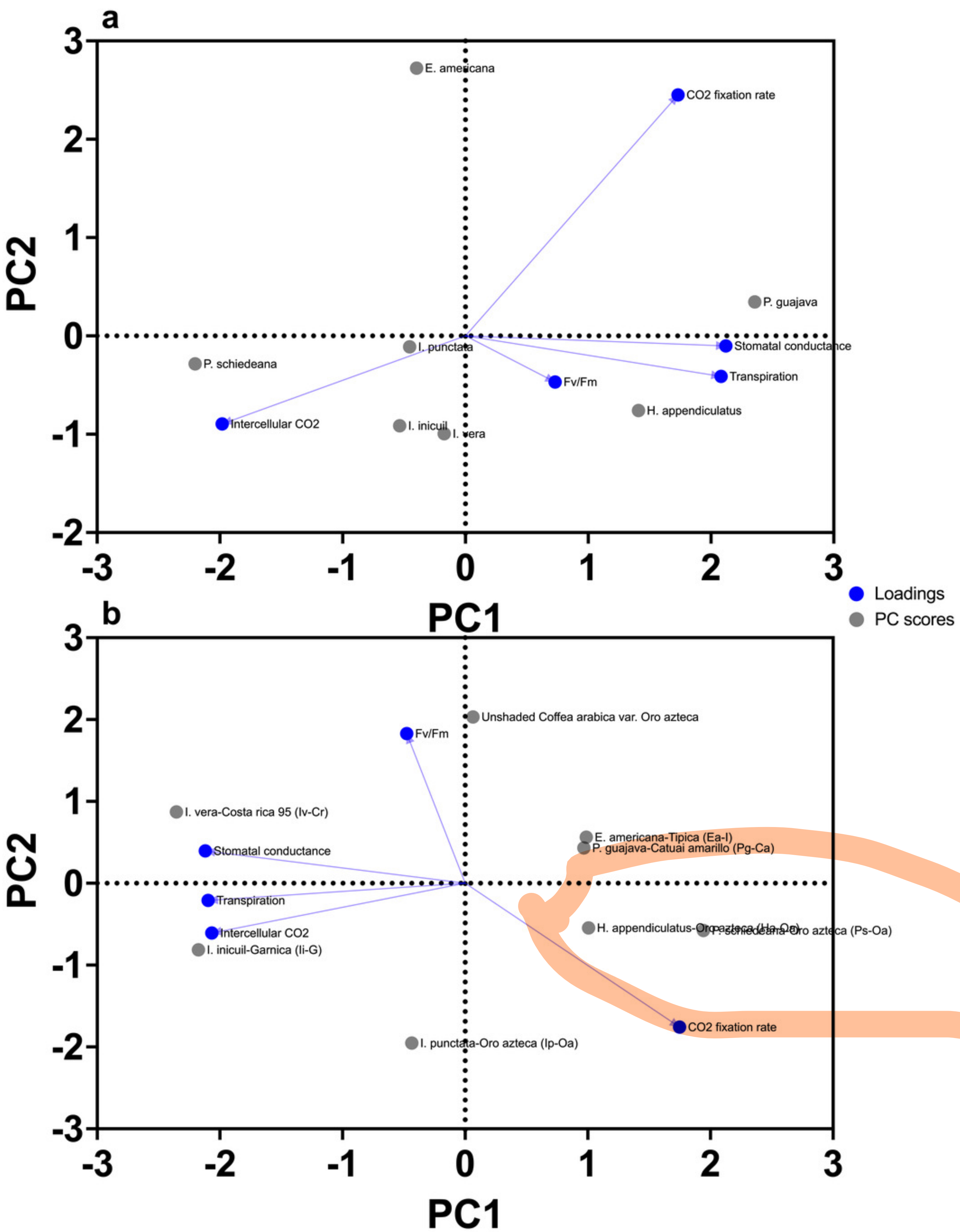
Mean ± SD (n=9). One-way ANOVA and a *post-hoc* Tukey's test were used to identify significant differences. Comparisons with a $p$ value ≤ 0.001 (***) are depicted.



# Figure 6

Principal component analysis (PCA) of chlorophyll fluorescence and gas exchange parameters.

(a) shade tree species; and (b) Arabica coffee varieties. PC1 represents 43.57% and 49.04% of the total variation for shade trees and Arabica coffee varieties, respectively, while PC2 represents 33.43% and 29.98% of the total variation for shade trees and Arabica coffee varieties, respectively (77% and 79.02% of the total variance for shade trees and Arabica coffee varieties, respectively). Correlation of variables with PCA axes is indicated by blue solid line vectors.


a
PC2
PC1
E. americana
CO2 fixation rate
P. guajava
I. punctata
Stomatal conductance
P. schiedeana
Fv/Fm
Transpiration
H. appendiculatus
Intercellular CO2
I. inicuil
I. vera
3
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
Loadings
PC scores
b
PC2
PC1
Unshaded Coffea arabica var. Oro azteca
Fv/Fm
I. vera-Costa rica 95 (Iv-Cr)
E. americana-Tipica (Ea-I)
P. guajava-Catuai amarillo (Pg-Ca)
Stomatal conductance
Transpiration
H. appendiculatus-Oro azteca (Ha-Oa)
P. schiedeana-Oro azteca (Ps-Oa)
Intercellular CO2
I. inicuil-Garnica (Ii-G)
CO2 fixation rate
I. punctata-Oro azteca (Ip-Oa)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3

## Table 1(on next page)

Dendrometric parameters of shade trees.

According with their carbon stock in biomass at 10 years, species are arranged descending. The average of three replicates is presented.

| **Tree specie** | **DBH[1] (m)** | **Height (m)** | **Age (years)** | **Allometric equation** | **AGB[2] (kg tree$^{-1}$)** | **Carbon stock[3] (kg tree$^{-1}$)** | **10-years carbon stock (kg tree$^{-1}$)** |
|---|---|---|---|---|---|---|---|
| *Persea schiedeana (Ps)* | 0.35 ± 0.05 | 14.33 ± 2.08 | 10.00 ± 4.58 | Exp((−3.1141)+((0.9719)*(Ln(DBH^2*H)) | 585.93 ± 124.85 | 275.38 ± 58.68 | 275.38 ± 58.68 |
| *Erythrina Americana (Ea)* | 0.58 ± 0.05 | 11.67 ± 2.02 | 30.00 ± 0.00 | [0.3700]*[DBH^1.9600] | 1054.91 ± 175.26 | 495.81 ± 82.37 | 165.27 ± 27.46 |
| *Inga inicuil (Ii)* | 0.24 ± 0.00 | 12.00 ± 3.46 | 10.00 ± 0.00 | [Exp[−1.76]*[DBH^2.26]] | 223.29 ± 3.46 | 104.95 ± 1.63 | 104.95 ± 1.63 |
| *Heliocarpus appendiculatus (Ha)* | 0.37 ± 0.02 | 15.67 ± 0.58 | 20.00 ± 0.00 | [[Exp[4.9375]]*[[DBH^2]^1.0583]]*[1.14]/1000 | 331.76 ± 30.59 | 155.92 ± 14.38 | 77.96 ± 7.19 |
| *Psidium guajava (Pg)* | 0.08 ± 0.01 | 5.00 ± 0.00 | 6.00 ± 1.73 | [0.246689]*[DBH^2.24992] | 28.25 ± 6.71 | 13.28 ± 3.15 | 22.13 ± 5.25 |
| *Inga vera (Iv)* | 0.10 ± 0.02 | 5.00 ± 1.00 | 11.67 ± 2.89 | [Exp[−1.76]*[DBH^2.26]] | 30.00 ± 13.21 | 14.10 ± 6.21 | 12.09 ± 5.32 |
| *Inga punctata (Ip)* | 0.07 ± 0.00 | 4.00 ± 0.00 | 13.33 ± 5.77 | [Exp[−1.76]*[DBH^2.26]] | 15.33 ± 0.78 | 7.20 ± 0.37 | 5.40 ± 0.27 |

[1] Diameter at breast height (1.30 m).

[2] Above-ground biomass.

[3] The carbon stock was determined by multiplying the calculate AGBs by the default carbon fraction of 0.47 set by the IPCC (2006).

# Table 2(on next page)

Carbon capture of shade tree species.

Estimated carbon stock per hectare using reported densities of the target shade trees species.

| Tree specie | ≈Trees ha$^{-1}$ | Carbon stock per hectare (kg C ha$^{-1}$) | Reference (Trees ha$^{-1}$) |
|---|---|---|---|
| *Persea schiedeana (Ps)* | 40 | 11015.2 | Soto-Pinto et al. 2001 |
| *Erythrina Americana (Ea)* | 40 | 19832.4 | Soto-Pinto et al. 2001; Garza-Lau et al. 2020 |
| *Inga inicuil (Ii)* | 200 | 20990 | Barradas and Fanjul, 1986 |
| *Heliocarpus appendiculatus (Ha)* | 40 | 6236.8 | Soto-Pinto et al. 2001; Romero-Alvarado et al. 2002 |
| *Psidium guajava (Pg)* | 40 | 531.2 | Somarriba, 1988; Akter et al. 2022 |
| *Inga vera (Iv)* | 200 | 2820 | Garza-Lau et al. 2020 |
| *Inga punctata (Ip)* | 100 | 720 | Valencia et al. 2014; Soto-Pinto et al. 2001 |
| **Total** | 660 | 62145.6 | |