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

First 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 and introduced woody vegetation enhance climate adaptation and mitigation by improving coffee farm resilience, carbon storage, and income diversification. Previously, based on ecosystemic, cultural, and economic criteria, seven native shade tree species were identified as shade providers in coffee agroforestry systems in Veracruz, Mexico . This study evaluated their physiological performance through above-ground biomass, carbon stocks, and *in-situ* chlorophyll fluorescence and gas exchange parameters under controlled conditions, alongside their effects on five common Arabica coffee varieties.

Erythrina americana and *Persea schiedeana* exhibited the highest carbon sequestration per tree. *Erythrina americana* showed higher water-use efficiency (16.92 µmol CO_2/mol H_2O), while *Persea schiedeana* had high stomatal conductance but drought vulnerability. In contrast, *Inga inicuil* achieved the highest carbon capture per hectare (198 Mg C ha^{-1}) due to its high planting density (660 trees ha^{-1}), despite lower individual tree performance . Physiological analyses revealed distinct functional groups. *Psidium guajava* and *Persea schiedeana* acted as stomatal regulators, with high transpiration but limited carbon gain. *Erythrina americana* and *Inga punctata* formed a drought-resilient group, prioritising carbon fixation over water loss. Intermediate species (*Heliocarpus appendiculatus*, *Inga vera*, *Inga inicuil*) balanced moderate photosynthesis with environmental adaptability.

Photochemical efficiency (Fv/Fm) remained stable across species (0.74–0.81) , though *Erythrina americana* showed slightly reduced values, possibly due to lower intercellular CO_2.

Shade trees significantly improved coffee performance. Shaded *Coffea arabica* var. Oro Azteca exhibited higher leaf nitrogen (2.83% vs 2.54% in unshaded), moisture content (55.56% vs 53.97%), and water-use efficiency (21.62 vs 4.61 µmol CO_2/mmol H_2O). PAR levels under *Persea schiedeana* and *Psidium guajava* were 30–169 times lower than unshaded coffee, suggesting enhanced long-term coffee productivity and photoprotection in shade.

Principal Component Analysis revealed key trade-offs: shade tree PC1 (43.6% variance) correlated with stomatal conductance, while PC2 (33.4%) showed an inverse relationship between CO_2 fixation and internal CO_2. Coffee plants exhibited opposite stomatal behaviour, with shaded varieties reducing transpiration despite higher conductance. High-density *Inga inicuil* maximises short-term carbon storage, while *Erythrina americana* enhances long-term resilience. *Persea schiedeana* offers superior microclimate regulation but requires careful management to prevent over-shading. These findings provide physiological criteria for selecting shade trees based on local climate and production goals, optimising Veracruz agroforestry systems for carbon sequestration, productivity, and sustainability.

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Abstract

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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, historically resistant to 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 native tree biomass (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 plays a crucial role in CO_2 mitigation through carbon assimilation and biomass storage (Fini et al., 2023; Jin et al., 2023). Accurate carbon sequestration estimates require above-ground biomass measurements via allometric equations, this calculation provides a realistic estimate of the amount of C assimilated into forest biomass and, consequently, of the function of carbon cycles in ecosystems (Liang and Wang, 2020; Araza et al., 2022). Physiological assessments, particularly leaf gas exchange and chlorophyll fluorescence analyses, provide vital insights into photosynthetic efficiency and plant responses to environmental changes (Genty et al., 1989; Sakshaug et al., 1998; Roháček, 2002; Baker and Rosenqvist 2004; Strasser, 2004; Baker, 2008), improving our understanding of ecosystem carbon dynamics. Shade trees in agroforestry systems critically modulate microclimatic conditions, directly influencing the physiological responses of coffee plants and associated tree species. Evidence indicates that shade reduces air temperatures by 1–5 °C, mitigating heat stress and maintaining optimal leaf temperatures (20–24 °C) for photosynthesis (Vaast et al., 2005; Lara-Estrada et al., 2023). This enhances chlorophyll fluorescence parameters such as Fv/Fm (quantum yield of PSII) by reducing photodamage under excessive irradiance (Rodriguez-López et al., 2014). However, shade-adapted leaves often exhibit lower heat tolerance than sun leaves, as observed in tropical trees like *Inga spectabilis* (Vahl) Willd., where shade leaves showed a reduced threshold for PSII dysfunction (Slot et al., 2019). Thus, while shade buffers chronic heat stress, it may not fully prepare plants for acute temperature extremes. Light availability also mediates leaf gas exchange in coffee plants. Shade levels of 30–50% optimise photosynthesis by balancing 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 refining these responses (Sauvadet et al., 2019; Isaac et al., 2024). Water availability under climate change is another key concern for agricultural and natural ecosystems. Water-use efficiency (WUE), defined as the CO_2/H_2O ratio during gas exchange (Kirkham, 2005), links plant productivity to water management (Bhattacharya, 2019; Hatfield and Dold, 2019). WUE varies with species traits and environmental conditions (Chaves et al., 2004; McCarthy et al., 2011). Shade improves WUE by increasing humidity (10–20%) and reducing stomatal conductance, though shaded coffee plants may show 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). The above evidence highlights the benefits of shade in stabilising microclimates and improving photosynthetic efficiency, but species-specific responses and trade-offs require further elucidation.

Therefore, the present work investigates the physiological characteristics of seven previously selected shade trees species and five *C. arabica* varieties that make up agroforestry systems in central Veracruz, Mexico. For this task, it was performed *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 under controlled temperature and Photosynthetic Photon Flux Density (PPFD) conditions.

We hypothesised that the observed physiological characteristics and interactions between these most-suitable seven tree species screened by Flores-Ortiz et al. (2025) and coffee varieties growing in shaded environments contribute to an improved carbon capture potential and coffee agroforestry system’s resilience to withstand climate change and coffee production over the long term by reducing environmental stress through shading and improving 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

The first seven native tree species were selected from an initial screening of 50 made by Flores-Ortiz et al. (2025) based on the level of conservation concern, their growth rate, and functionality for coffee growers, excluding species used as firewood or fuel, as well as *Persea americana* Mill. (Hass Avocado) due to its intensive domestication. Dendrometric, chlorophyll fluorescence and gas exchange characteristics of the 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*), were measured in trees ≤30 years old.

Due to the heterogeneity of *C. arabica* varieties found in the coffee farms in the region, chlorophyll fluorescence and gas exchange parameters tested were restricted to Oro Azteca (Oa), Garnica (G), Costa Rica 95 (Cr), Tipica (T), and Catuai amarillo (Ca), in bushes with ages between 4–6-years. For comparative purposes, measurements of the different response parameters were also performed in bushes of Oro Azteca variety 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 bushes in unshaded conditions were part of an unshaded monoculture system. The tree density, considering trees between 10–15 m 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 in ≈1000 trees ha^{-1} (López-Gómez et al., 2008; Williams-Linera and Lorea, 2009).

Agroforestry systems were located in central Veracruz on a 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). Climate data for this period matched typical 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 farms share similar 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 used 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 into biomass carbon stock (CS) by multiplying AGB by 0.47, which represents the carbon fraction linked to the computed biomass (IPCC, 2006). CS is associated with the tree's ability to grow new cells and 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 included in this study. Normalisation to a common age baseline thus allows for a more standardised assessment of carbon storage potential across species, while still acknowledging that species-specific physiological adaptations, such as shade tolerance, wood density, may influence long-term sequestration trends.

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

Measurements were conducted using a portable Infrared Gas Analyser (IRGA) Model LI-6400XT (LI-COR, Inc.) equipped with a fluorometric cell and conducted on three fully developed shade tree leaves attached on the first lateral branches at the base of the trunk (branches closest to the understory layer), between 2–4 m from the ground. These branches exhibited sympodial growth with a predominantly horizontal or obliquely oriented 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 bush measurements were taken from leaves attached to plagiotropic branches at 1.30–2 m height.

Chlorophyll fluorescence analysis were performed to leaves after 30 minutes of dark adaptation by foil wrapping of the leaf. Subsequently, leaves were exposed to 650 nm light with an intensity of 2000 µmol m^{-2} s^{-1} for 2 seconds, and the values of the fluorescence emitted by the chlorophyll of PSII were measured and stored simultaneously. Basal or initial fluorescence (F0) and maximum fluorescence (Fm) were recorded and subsequently, the variable fluorescence at 2 µs (Fv, difference between Fm and F0) and quantum yield (Fv/Fm) were calculated. Fv/Fm is frequently used to estimate the photochemical efficiency of PSII (Niinemets and Kull 2001; Lepeduš et al., 2005; Zavafer and Mancilla, 2021). All measurements were conducted under controlled conditions, with this aim, the IRGA was operated as open system with a photon flux density of 1000 µmol m^{-2} s^{-1}, leaf temperature 25º C, and at environmental CO_2 concentration. After that, the gas exchange parameters CO_2 fixation rate, stomatal conductance, transpiration, intercellular CO_2 (Ci), were measured in the same leaves attached on the branch.

After completing the chlorophyll fluorescence and gas exchange tests, it was 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 bushes at midday; this period sees peak solar radiation and thermal stress (Meili et al., 2021; Kohl et al., 2024).

Finally, instantaneous leaf WUE or transpiration efficiency was calculated as follows (Hatfield and Dold, 2019):

$$WUE = \frac{A}{E} \tag{1}$$

Where *WUE* is the instantaneous leaf Water-Use Efficiency expressed as µmol CO_2/mmol H_2O, *A* is CO_2 Fixation Rate expressed as µmol CO_2 m^2/s, and *E* is Transpiration Rate expressed as mmol H_2O m^2/s.

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

Immediately following the measurements of chlorophyll fluorescence and leaf gas exchange, the same leaves from Oro Azteca coffee plants grown under both shaded and unshaded conditions were excised at the base of the petiole. To minimise excessive moisture loss, the collected leaves were placed in sealed plastic containers 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:

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

where *H (%)* is the moisture content expressed as a percentage, *FW* is the fresh weight and *DW* is the dry weight.

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

Statistical analysis

In all cases, analyses 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. Shade trees and coffee data met assumptions of normality, so no transformations were needed.

One-way ANOVA and the *post-hoc* Tukey test were used to identify significant differences ($p < 0.033$). 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.

Statistical analyses of Oro Azteca moisture and nitrogen content in shaded conditions vs unshaded conditions were performed using a two-tailed unpaired t-test (t (7.6) = 4 and 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 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 40 trees of *Ea*, *Ps,* and *Ha* represent 94.48%, 52.47%, and 29.71%, respectively, of the carbon assimilated by 200 individuals by *Ii*, the species with 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 bushes (0.73–0.80) showed consistent trends, with no significant differences observed (Fig. 2a, Table S1). Similarly, no significant differences were found between shaded and unshaded Oa or among other coffee varieties under shaded conditions (Fig. 3a and 4a, Table S2).

The CO_2 fixation rate varied significantly among species ($F_{(6, 56)} = 118.3$, $p = 0.001$; $p < 0.033$), with the highest rates observed in *Ea*, *Pg*, and *Ha*, followed by *Ip*, and then *Ps*, *Iv*, and *Ii* (Fig. 2b, Table S1). Regarding the Oa coffee variety, CO_2 fixation rates differed significantly between shaded and unshaded conditions $F_{(3, 32)} = 54.42$, $p = 0.001$; $p < 0.033$). Among shaded coffee varieties, *Ip*-Oa and *Ha*-Oa exhibited the highest CO_2 fixation rates, while other combinations showed lower values ($F_{(6, 56)} = 16.82$, $p = 0.001$; $p < 0.033$) (Fig. 3b and 4b, 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, $p = 0.001$; $p < 0.033$) (Fig. 2c, 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, $p = 0.001$; $p < 0.033$) (Fig. 3c, Table S2). Among shade-grown coffee varieties, *Ii*-G demonstrated the highest transpiration rates, while *Ea*-T, *Ha*-Oa, and *Ps*-Oa exhibiting the lowest (F $_{(6, 56)}$ = 17.98, $p = 0.001$; $p < 0.033$) (Fig. 4c, Table S2).

Stomatal conductance exhibited patterns similar 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, $p = 0.001$; $p < 0.033$), particularly between the highest and lowest performers (Figure 2d, 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, $p = 0.001$; $p < 0.033$) (Fig. 3d, 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, $p = 0.001$; $p < 0.033$) (Fig. 4d, 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, $p = 0.001$; $p < 0.033$) (Fig. 2e, 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, $p = 0.001$; $p < 0.033$) (Fig. 3e, 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, $p = 0.001$; $p < 0.033$) (Fig. 4e, Table S2).

The PAR measurements recorded at midday revealed significant variation 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, $p = 0.001$; $p < 0.033$) (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, $p = 0.001$; $p < 0.033$) (Table S2).

WUE values showed significant variation among the seven tree species studied, ranging from 0.83 ± 0.15 µmol CO_2/mmol H_2O in *Ii* to 16.92 ± 11.05 µmol CO_2/mmol H_2O in and *Ea* (F $_{(6, 56)}$ = 5.810; $p < 0.001$; $p < 0.033$). The highest WUE values were recorded 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 µmol CO_2/mmol

H_2O in unshaded conditions to 21.62 ± 11.27 µmol CO_2/mmol H_2O in the *Ha*-Oa shaded association (F $_{(3, 32)}$ = 8.538; $p < 0.001$; $p < 0.033$) (Fig. 5b, Table S2). Among shaded Arabica cultivars, values varied from 7.12 ± 3.78 µmol CO_2/mmol H_2O in *Iv*-Cr to 21.62 ± 11.27 µmol CO_2/mmol H_2O in *Ha*-Oa, with statistically significant differences observed (F $_{(6, 56)}$ = 4.315; $p < 0.001$; $p < 0.033$) (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 notable 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 potential role 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 agroforestry system C capture. 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 exhibit distinct photosynthetic traits and growth and biomass allocation patterns, leading to inherent variability in carbon accumulation and therefore 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.

Variation in carbon storage among the species reveals 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 superior 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 (20–40% of total trees) significantly contributed to carbon capture (Soto-Pinto et al., 2001), and including *I. punctata* and *I. vera* could achieve 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 what 200 *I. inicuil* trees achieve. However, biological constraints, such as 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 limit their densities in managed systems.

Third, the remaining species provide complementary functions. *H. appendiculatus*, found at 16–20% of tree strata in Chiapas coffee farms (Soto-Pinto et al., 2001; Castillo-Capitán et al., 2014), contributed 10.03% of total carbon assimilation despite representing only 6% of trees in this study. *P. guajava*, constituting 4–5% of tree density in coffee plantations (Soto-Pinto et al., 2001), had among 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 to 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, displaying an inverse relationship between CO_2 fixation rate and Ci, indicative of 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 superior 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) demonstrated consistently low stomatal conductance and CO_2 fixation under moderate temperatures (23.43 °C) and high relative humidity (70.4%), a response linked 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, 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 climate resilience. WUE analysis revealed reduced transpiration under high temperature and radiation conditions, prioritising water conservation, a trait advantageous for drought-prone agroforestry, though reliance on high humidity may limit performance in arid microclimates.

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 ± 0.38 to 5.02 ± 1.34 μmol CO_2 m^{-2} s^{-1}) compared to other *Inga* species under controlled conditions (10.60–11.65 μmol CO_2 m^{-2} s^{-1}; dos Santos Pereira et al., 2019), likely due to differences in measurement, such as mature leaves vs younger leaves, light intensity, 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, displayed higher CO_2 assimilation (3.7–11.6 μmol 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.

Photosynthetically active radiation (PAR) beneath canopies varied significantly: *E. Americana* and *Inga* species permitted 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) from 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, indicating 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), underscoring 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, underscoring the need for microclimate moderation to optimise productivity. Variability in CO_2 fixation rates between varieties also indicates differing 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). Contrary, shaded plants exhibit more stable CO_2 fixation rates due to moderated environmental conditions.

WUE in 14 *C. arabica* cultivars under drought ranged from 4–12.5 µmol CO_2/mmol H_2O (Reis et al., 2022), while our shaded *H. appendiculatus*-Oro Azteca variety achieved 21.62 µmol CO_2/mmol H_2O, double that of drought-tolerant genotypes. Traits like thicker cuticles, fewer xylem vessels, and higher stomatal density enhanced WUE (Baliza et al., 2012; Wolfe et al., 2016). Seasonal gas exchange in 21 genotypes revealed stable WUE (1.2–3.4 µmol CO_2/mmol H_2O; Tezara et al., 2022), generally lower than our values except for unshaded Oro Azteca bushes.

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 bushes 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, and environmental conditions assessed. 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.

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* excels in carbon storage per tree, combining high WUE with CO_2 fixation. In contrast, *I. inicuil* dominates hectare-scale carbon capture due to its high density, illustrating the trade-off between individual tree efficiency and population-level impact. These distinctions underscore the need for strategic species selection, prioritising *I. inicuil* for rapid carbon storage and *E. americana* and *P. schiedeana* for long-term resilience.

Functional groupings reveal complementary adaptations: *P. guajava* and *P. schiedeana* optimise photosynthesis in moist environments, whereas *E. americana* and *I. punctata* conserve water while maintaining carbon uptake. Intermediate species like *H. appendiculatus* and *I. vera* balance 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.

Concerning 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 bushes under *H. appendiculatus* 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 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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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 punctate (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 punctate (Ip)	100	720	Valencia et al. 2014; Soto-Pinto et al. 2001
Total	660	62145.6	

1

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.

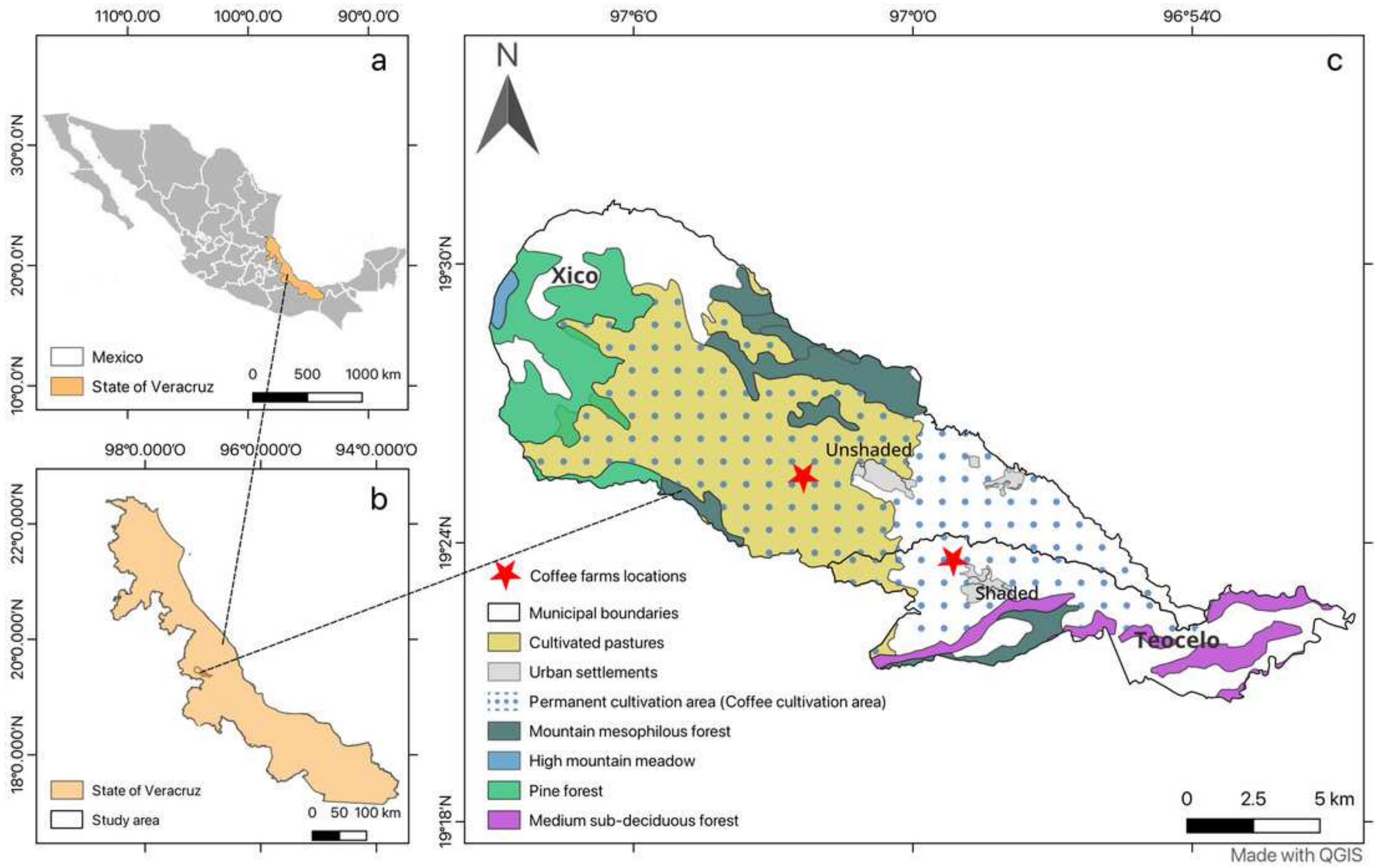

Figure 2

Photosynthetic and 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. Only comparisons with a P values ≤ 0.001 (***) are depicted.

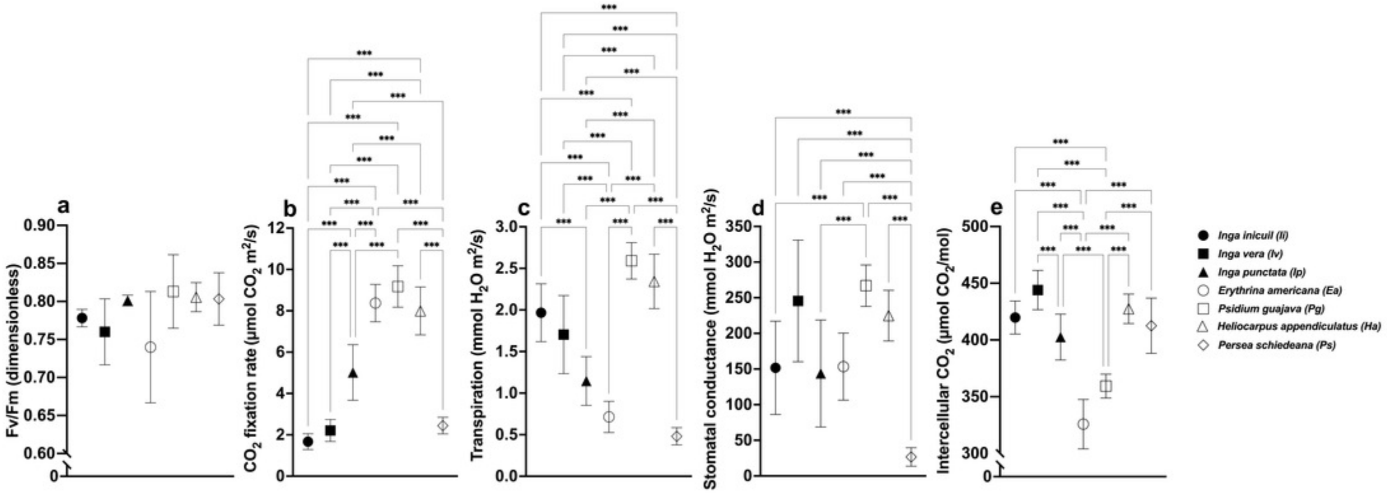

Figure 3

Photosynthetic and 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. Only comparisons with a P value ≤ 0.001 (** , ***) are depicted.

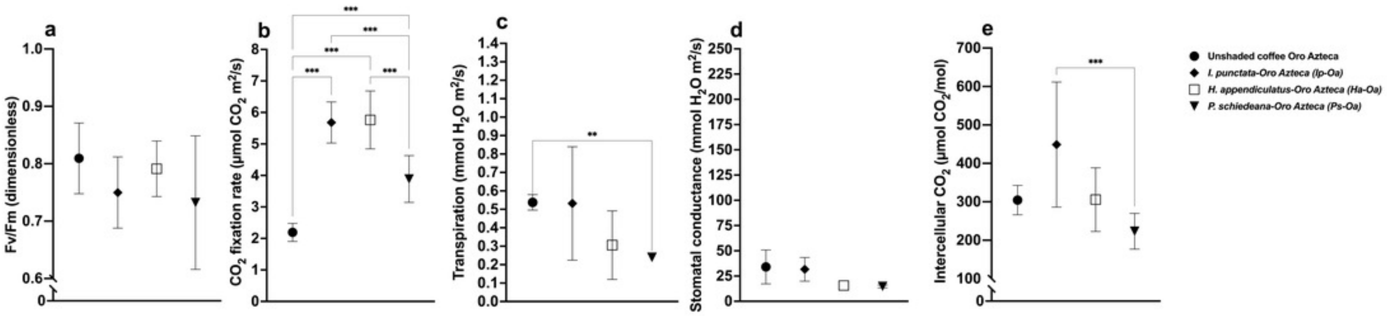

Figure 4

Photosynthetic and 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. Only comparisons with a P value ≤ 0.001 (***) are depicted.

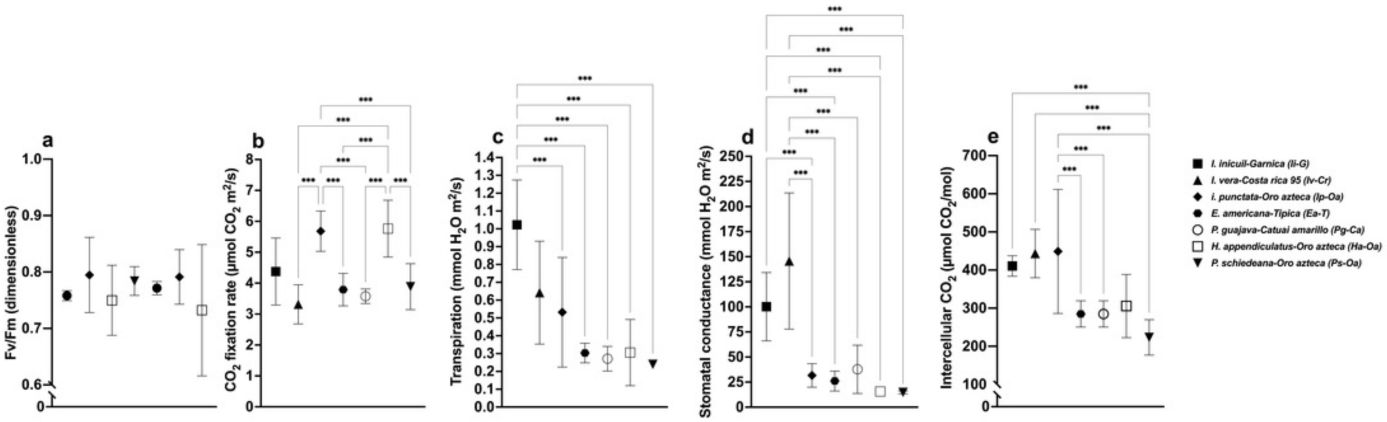

Figure 5

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. Only comparisons with a P value ≤ 0.001 (***) are depicted.

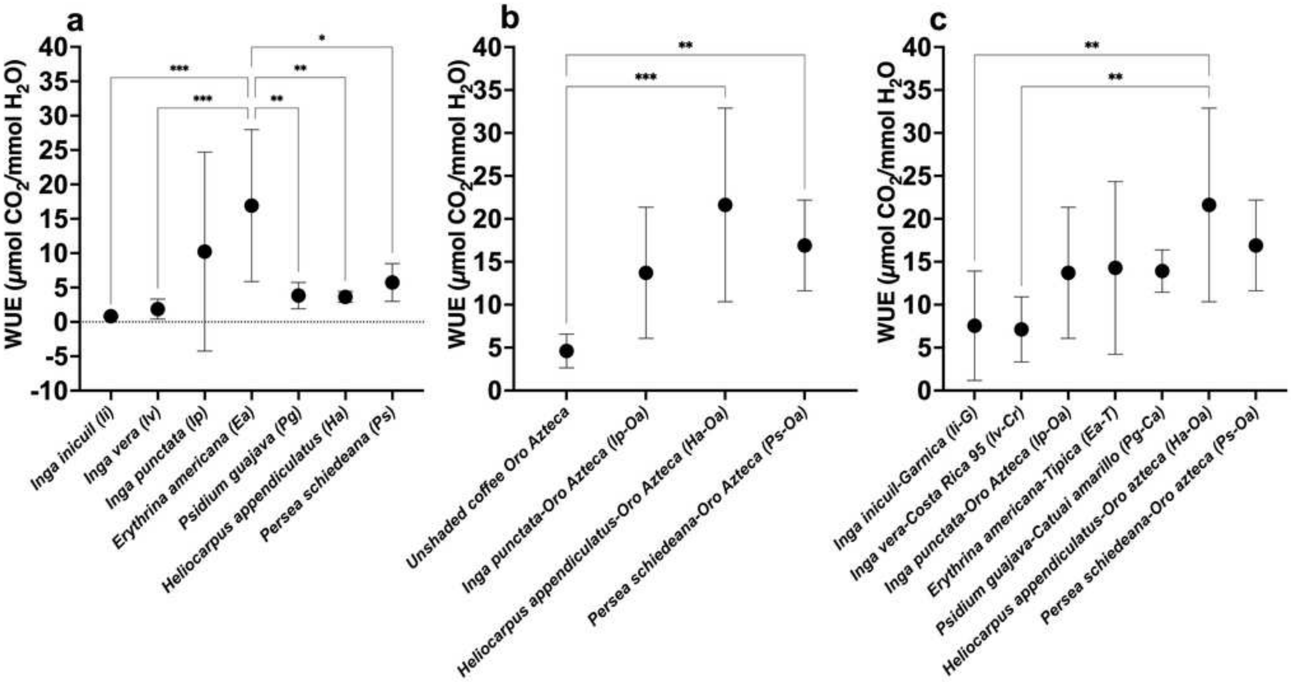

Figure 6

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.