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

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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 contribute to climate adaptation and mitigation by enhancing coffee farm resilience, storing carbon, and diversifying farmers' income. This study explores how native tree species and coffee varieties in shaded environments reduce exposure to environmental stress, enhance carbon capture, and strengthen agroforestry resilience against climate change. Previously, seven native tree species were identified as optimal shade providers in coffee agroforestry systems in Veracruz, Mexico, based on ecosystemic, cultural, and economic criteria. This study analysed above-ground biomass, carbon stocks, chlorophyll fluorescence, leaf gas exchange, and leaf water-use efficiency in these trees and five Arabica coffee varieties. *Persea schiedeana* and *Inga punctata* exhibited contrasting carbon capture values (275.38 and 5.40 kg tree^{-1}, respectively), yet all tree species displayed high resilience to irradiation stress (0.73–0.80 Fv/Fm). Regarding photosynthesis and gas exchange, *Erythrina americana* had the highest photosynthetic rates, while *Inga inicuil* had the lowest. Transpiration varied significantly, with *Psidium guajava* showing the highest and *Persea schiedeana* the lowest rates. A similar pattern was observed in stomatal conductance. *Inga vera* had the highest intercellular CO_2 concentration, whereas *Erythrina americana* had the lowest. Leaf water-use efficiency was highest in *Erythrina americana* and *Inga punctata*. Understory photosynthetically active radiation levels varied widely, with unshaded conditions reaching up to 169 times higher than shaded environments. The results suggest that *Persea schiedeana* and *Erythrina americana* provide significant climate resilience due to their physiological traits and carbon storage potential. Although *Inga vera*, *Inga punctata*, *Heliocarpus appendiculatus*, and *Psidium guajava* showed lower individual carbon capture, their high tree density and economic benefits make them valuable components of agroforestry systems. Regarding coffee varieties, Oro Azteca exhibited significant differences in CO_2 fixation under unshaded and shaded conditions. However, its Fv/Fm values remained stable, indicating resilience of PSII under high photosynthetically active radiation conditions. Transpiration, stomatal conductance, and intercellular CO_2 concentrations were generally higher in shaded coffee varieties under *Inga* spp. canopies than in unshaded conditions. Additionally, shaded varieties demonstrated increased CO_2 fixation under elevated photosynthetically active radiation. Shaded Arabica varieties had higher leaf water-use efficiency compared to unshaded counterparts, emphasizing the role of shade in optimising water use. These varieties also exhibited greater moisture and nitrogen content, improving productivity and photoprotection. This study highlights the diverse ecophysiological adaptations within coffee agroecosystems and the physiological advantages conferred by shade cover.

Abstract

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Subjects: Biodiversity, Conservation Biology, Natural Resource Management, Climate Change Biology; Environmental Impacts, .

Keywords: Agroforestry systems; Carbon capture; Leaf gas exchange; Photosynthesis, Water-use efficiency; Smallholder coffee farmers; Chlorophyll fluorescence; Climate resilience, Shade Coffee; *Coffea arabica* varieties.

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), ~~only petroleum and its derivatives are more significant legal export commodities than coffee (Toledo and Moguel, 2012).~~ 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). The 70% of global coffee production was exported in 2017 with a value of USD $19 billion and provided employment for 125 million people worldwide (Fairtrade Foundation, 2022; Panhuysen and Pierrot, 2020). Although the coffee industry was valued at US$132.13 billion in 2024, the majority of the profits made throughout the value chain are concentrated in the post-production phase of the value chain, benefiting retailers, processors, and exporters, not small producers (Mordor Intelligence, 2024). These producers also suffer from limited access to market facilities, less exposure to market information, infrastructure issues, insufficient support services, and transportation issues (Bizualem, 2015).

Currently, there are 12.5 million coffee farms worldwide, and the majority which are located in regions identified as important biodiversity zones (Donald, 2004) and owned by smallholders with less than 5 hectares (Fairtrade Foundation 2022; Panhuysen and Pierrot 2020). In Mexico, coffee is produced on approximately 580,000 ha by 481,000 farmers and well over a million people depend on coffee for their livelihoods (Ellis et al., 2010; Harvey et al., 2021; USDA FAS, 2023). Approximately 86% of the coffee produced in Mexico is *Coffea arabica*, and 90% of farms are smaller than 1.5 ha. Most of these farms are shade polycultures with mixes of native and introduced tree species and vegetation structures that create habitat for biodiversity and provide important ecosystem services (Beer et al.,1998; Moguel and Toledo 1999; Dávalos-Sotelo et al., 2008; Toledo and Moguel 2012; Jha et al., 2011).

While coffee production in Mexico has traditionally resisted intensification (Rice, 1999), current crises in production due to climate change have pushed growers in this direction or even the complete conversion of coffee farms to other land uses (Harvey et al., 2021). Alternative land uses such as agriculture and other industries, constituted over 23% for North America and 11% for Latin America and Caribbean of the net anthropogenic greenhouse gas (GHG) emissions during the period from 1850–2019 (IPCC, 2022), directly influencing climatic conditions. Coffee production is affected by changes in temperature, humidity, and rainfall that trigger phenological changes during growth, making it one of the crops most sensitive to climate change (Pham et al., 2019; Bilen et al., 2023), with a reduction in the growing area under most climate change scenarios (Bunn et al., 2015). Furthermore, these types of transitions have negative impacts on biodiversity (Toledo and Moguel, 2012), and a number of important ecosystem services (Beer et al., 1998; Davidson, 2004; García-López, 2009; Adugna and Struik, 2011; Hausermann, 2014; Harvey et al., 2021).

A growing number of studies explore how to utilise the synergies between adaptation and mitigation in agroforestry systems like shade coffee to help growers meet the challenges of climate change through atmospheric GHG reduction (up to 0.31 Pg C year^{-1}) and associated carbon credits, soil and biodiversity conservation, or diversification of production (Verchot et al., 2007; Rahn et al., 2014; Jawo et al., 2022; Terasaki Hart et al., 2023). One possible sustainable strategy for increasing carbon (C) capture based on standing plant biomass is the use of native, perennial tree species (Noponen et al., 2013) with multiple uses for farmers (Jha et al., 2011). These systems can also produce fruit, medicines, or lumber for additional income. Additionally, agroforestry systems can buffer temperatures of the air and soil, as well as increase humidity and the availability of water and nutrients in the soil (Segura et al., 2006; Lin, 2007; 2010; Siles et al., 2012; Notaro et al., 2014; Ehrenbergerová et al., 2018). Thus, adaptation efforts involving an increase of shade cover to reduce the effects of climate change on coffee production may simultaneously increase biomass and C capture and thus mitigate GHG emissions while conserving endangered native tree species (Acevedo et al., 1992; Jose, 2009; Häger, 2012; Noponen et al., 2013; Gross et al., 2022).

~~Adaptation and mitigation efforts should consider the conservation of biodiversity and ecosystem services an the socioeconomic well-being of farmers, social justice, and economic equity (Guzman-Luna et al., 2022).~~ Aspects to be considered in selecting tree species for shade tree diversification in coffee farms include improving soil fertility, optimising productivity, and generating alternative sources of income (Gliessman, 2015); prioritising those species that help coffee bushes retain moisture in the soil, control pests and diseases, and avoid the introduction of exotic species that could trigger various types of physicochemical and biotic stresses, decreasing vegetation diversity and understorey flora (Reigosa et al., 2000; Gill and Prasad 2000).

Due to the transition between the Nearctic and Neotropical regions (Rzedowski, 1978), the Mexican state of Veracruz has a wide range of environmental variations and a high biological diversity, making it one of the most biologically diverse states in the country (Estrada-Contreras et al., 2015) and according with Tellez et al. (2020), the state is one of the two tree biodiversity hotspots in Mexico. ~~As such, it is a relevant case study to understand the function, effects, and synergies of agroforestry systems in developing countries facing climate change and mitigation from a multidisciplinary point of view~~. Since Veracruz coffee-producing communities rank first in the country, accounting for 23% of the national total yield (Nestel, 1995), as well as the importance of the Veracruz agroforestry systems as carbon sinks, a variety of approaches have been used to estimate their C capture potential. For instance, the carbon-storing capability of coffee agroecosystems in the municipality of Xalapa, Veracruz, was quantified, finding that the combination of coffee bushes and trees taller than 15 m had a higher C storage capability (73.27 Mg C ha^{-1}) compared with agroforestry systems comprising coffee and the Fabaceae *Acacia pennatula* Schltdl. & Cham. (38.47 Mg C ha^{-1}) (Ortiz-Ceballos et al., 2020). The highest ratio of total C storage (91.2%) was measured in coffee combined with trees >15 m in height, and coffee bushes combined with *Inga* sp (Ortiz-Ceballos et al., 2020). Additionally, research on the relationship between C and biodiversity throughout a gradient of intensified land usage was conducted in the municipality of Xalapa, finding that biodiversity and C storage are positively correlated, with C being highest in forests; however, secondary forests and conventional shade coffee plantations shared similar characteristics (Dávalos-Sotelo et al., 2008; Vizcaíno-Bravo et al., 2020).

Plant-based CO_2 mitigation, such as carbon assimilation by photosynthesis and its final storage into trees biomass, is expected to play a key role in mitigating the atmospheric release of CO_2 and other GHG by industrialization and urbanisation (Fini et al., 2023; Jin et al., 2023). Estimates of carbon sequestration include measures of above-ground biomass (AGB) through 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). Furthermore, plant photosynthetic activity is crucial during the early stages of removing carbon from the atmosphere (IPCC, 2021). Consequently, evaluations of the physiological responses of trees and shrubs to environmental factors that impact C capture potential and the available substrates for photosynthesis through leaf gas exchange analyses are important because they are associated with both climatic change and the functioning characteristics of ecosystems (Sakshaug et al., 1998; Roháček, 2002). Furthermore, measurements of chlorophyll fluorescence are among the most widely used methods in plant physiology due to the ease with which one can obtain comprehensive data regarding the functionality and efficiency of photosystem II (PS II). This parameter provides important insights into the basic processes of photosynthesis, genetic variation, ecological diversity, and plant responses to environmental change (Genty et al., 1989; Strasser, 2004, Baker and Rosenqvist 2004; Baker, 2008).

Another major concern of climate change is water availability and how agricultural and natural ecosystems cope with limited water resources. Water-use efficiency (WUE) links plant productivity and water use, offering a valuable parameter to assess species' ability to manage water resources and the impact of climate change on plant growth and water use (Bhattacharya 2019; Hatfield and Dold, 2019). During gas exchange, plants fix atmospheric CO_2 into biomass while losing water vapour through evapotranspiration. Thus, WUE is defined as the amount of water a plant consumes or loses to incorporate a given amount of CO_2, expressed as the ratio of CO_2/H_2O (Kirkham, 2005). WUE is influenced by species and environmental conditions (Chaves et al., 2004), with variations due to factors such as leaf phenology, shade tolerance, successional status, and rooting patterns (McCarthy et al., 2011). ~~Modern portable gas analysers now allow leaf-level WUE measurements based on photosynthesis and transpiration rates, offering an effective approach to evaluating species' water management abilities (Kirkham, 2005).~~

Therefore, the present work was aimed to better understand the physiology of shade trees and how their shade affects coffee varieties, we conduct an *in-situ* measurement of the dendrometric, photosynthetic, and gas exchange parameters and leaf WUE calculation of seven previously selected native tree species (Flores-Ortiz et al. accepted for publication) and five Arabica coffee varieties that maximise conservation, carbon sequestration in central Veracruz state, Mexico. We hypothesised that native tree species and coffee varieties growing in shaded environments, are less exposed to stressful environmental conditions, and more capable of capturing carbon and improving coffee agroforestry system' resilience to withstand climate change and coffee maintain production over the long term. We expect that this information will help decision-making during the selection and management of suitable shade trees based on their physiological characteristics and C capture potential in shade coffee agroforestry systems.

Materials & Methods

Selection of shade tree species in coffee agroforestry systems

This research focussed on the top seven species from an initial list of 50 native tree species prioritised based on the level of conservation concern, their growth rate, and functionality for coffee growers (Flores-Ortiz et al. accepted for publication). Species used as firewood or fuel were excluded, as well as *Persea americana* Mill. (Hass Avocado) due to its intensive domestication (Table 1).

Study area

Field measurements were made in central Veracruz on a shaded and unshaded coffee farm located 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). Field measurements were taken during three consecutive days monthly (October-November 2022), during which temperature and RH were recorded by probes coupled to the Infrared Gas Analyser (IRGA) Model LI-6400 (LI-COR, Inc.). Average annual precipitation for these farms is 1.7 mm with minimal variation in climate due to their proximity. Measurements in both farms were conducted during October and November 2022.

Dendrometric parameters of shade trees and carbon stocks determination

Measurements were made of the total height and the diameter at breast height (DBH, at 1.30 m) of nine mature and reproductive individuals randomly selected of the seven species under shade. Using these morphometric parameters, available allometric equations were used to calculate each tree's AGB (Table 2). The allometric equations used to calculate the AGBs for *Heliocarpus appendiculatus* (*Ha*) and *Psidium guajava* (*Pg*) were at species-level, at genus-level for *Inga* spp. (*Inga inicuil Ii*, *Inga vera Iv* and *Inga punctata Ip*) and for *Erythrina* sp. (*Erythrina Americana Ea*), and at tropical forest-level for *Persea schiedeana* (*Ps*) (Rojas-García et al., 2015; Ortiz-Ceballos et al., 2020) (Table 2). 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 simple proportionality rule for each tree species included in this study.

Photosynthetic and gas exchange properties of shade trees and coffee plants

At midday, gas exchange analyses were conducted using the IRGA coupled with a fluorometric cell for photosynthetic analysis. Tree and coffee bush photosynthesis was evaluated using leaf chlorophyll fluorescence and gas exchange parameters under unshaded and shaded conditions (Sakshaug et al., 1998; Roháček, 2002). Three stomatal and three photosynthetic parameters were measured on fully developed shade tree leaves that were located on initial plagiotropic branches of three individuals' tree and coffee bushes per species or coffee variety. On each individual, three fully developed mature leaves were selected for chlorophyll fluorescence and gas exchange measurements. Collected data from each individual were averaged per shade tree, and per tree species or coffee variety. Parameters measured included Fv/Fm, also referred to as Genty's parameter (ratio of variable to maximal fluorescence), CO_2 fixation rate, stomatal conductance, transpiration, intercellular CO_2 (Ci), and photosynthetically active radiation (PAR) used to estimate incident solar radiation in the understory layer. The Fv/Fm ratio, a chlorophyll fluorescence parameter applied to dark-adapted samples, is used to calculate PSII maximum efficiency with all its reaction centres open. This efficiency estimates stress and plant photosynthetic performance (Lepeduš et al., 2005; 2012), via inhibition or damage to the electron transfer process in PSII (Niinemets and Kull 2001; Zavafer and Mancilla, 2021). After 30 minutes of dark adaptation by foil wrapping of the leaf, a pulse of saturating red light was applied to induce the chlorophyll fluorescence transient (peak at 650 nm, 1000 µmol m^{-2} s^{-1}); F0 and Fm were recorded and subsequently, Fv (difference between Fm and F0) and Fv/Fm were calculated.

Due to the heterogeneity of *Coffea arabica* varieties found in the coffee farms in the region, the measurements of the different parameters tested were restricted to Oro Azteca (Oa), Garnica (G), Costa Rica 95 (Cr), Tipica (T), and Catuai amarillo (Ca). For comparative purposes, measurements of the different response parameters were also performed in bushes of Oa in unshaded conditions.

To ensure homogeneity in environmental conditions, the measurements were made over a period of two consecutive cool-season months (October and November 2022). The climate data for these months aligns with typical conditions observed between 2016–2022, showing precipitation, temperature, and cloudiness within expected ranges (Weather Spark, 2024). This period marks the cool season, with average maximum temperatures of 24°C in Teocelo and 26°C in Xico. Precipitation for both municipalities was at least 1 mm/h and cloud cover averaged 80% (Weather Spark, 2024). Historical records from the National Meteorological Service indicate maximum and minimum temperatures in Teocelo of 35°C and 21°C (1945–2020), while Xico experienced a range of 21–22°C maximum and 9–12°C minimum temperatures (1966–2023) (Servicio Meteorológico Nacional, www.smn.conagua.gob.mx). PAR measurements under shade conditions were similar, thus ensuring the same shade conditions during data collection, as well as a clear contrast with the condition to which the coffee trees were exposed in unshaded conditions.

Instantaneous leaf water-use efficiency (ILWUE)

Instantaneous leaf WUE or transpiration efficiency was assessed through gas exchange measurements conducted through IRGA on the seven tree species and five Arabica varieties. Water-use efficiency on a leaf basis can be expressed as follows (Hatfield and Dold, 2019):

$$ILWUE = \frac{A}{E} \qquad (1)$$

Where *ILWUE* 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

Leaves collected for chemical analysis were quickly stored after photosynthesis and gas exchange analysis. Following *in-situ* sampling and drying, a 0.5 g of sample leaves from both unshaded and shaded coffee bushes were used to measure the moisture and nitrogen contents over the next two days. Gravimetric methods comparing weight before and after drying determined moisture content based on the following equation:

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

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

All analyses were performed in triplicate on mature trees and coffee bushes, and on fully developed leaves. Statistical analyses were conducted on averaged data. No comparisons were performed for the dendrometric parameters of the seven native tree species due to inherent genotypic and phenotypic variations among species. Shade trees and coffee bushes stomatal, photosynthetic and ILWUE 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, ($p < 0.033$). Multiple comparisons were conducted between the parameter values of native shade tree species. Evaluations of environmental stress levels between unshaded and shaded coffee bushes were evaluated using comparisons between Oa in both conditions. Statistical analyses of Oa moisture and nitrogen content under unshaded and shaded were performed using a two-tailed unpaired t-test (t (7.6) = 4 and p = 0.002). 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

Genotypic and phenotypic variations among the seven shade tree species significantly influence their growth patterns and biomass allocation, making interspecific AGB and CS data comparisons unrecommended ~~(Poorter et al., 2008; Chave et al., 2014).~~ 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 2).

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 3). 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 and one of those with the highest reported population.

Photosynthetic and gas exchange properties 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.

CO_2 fixation rate revealed clear groups among the species with the highest rates observed in *Ea*, *Pg*, and *Ha*, followed by *Ip*, and then *Ps*, *Iv*, and *Ii*, with significant differences between these clusters ($F_{(6, 56)} = 118.3, p = 0.001; p < 0.033$) (Fig. 2b, Table S1). The top-performing group showed CO_2 fixation rates substantially higher than the intermediate and lowest clusters (1.7-fold and 4.18-fold higher, respectively). Concerning Oa variety, CO_2 fixation rate in unshaded coffee bushes differed significantly versus shaded conditions ($F_{(3, 32)} = 54.42, p = 0.001; p < 0.033$), further highlighting the variability in CO_2 fixation performance under both conditions. Shaded coffee varieties also formed distinct clusters, with *Ip*-Oa and *Ha*-Oa exhibiting 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 also varied significantly among tree species and coffee varieties. The highest rates were observed in *Pg* and *Ha*, while intermediate rates were seen in *Ii* and *Iv*, and the lowest in *Ip*, *Ea*, and *Ps* ($F_{(6, 56)} = 65.43, p = 0.001; p < 0.033$) (Fig. 2c, Table S1). Among coffee Oa, unshaded and shaded *Ip*-Oa combinations showed the highest transpiration, while *Ha*-Oa and *Ps*-Oa had the lowest ($F_{(2, 32)} = 6.488, p = 0.001; p < 0.033$) (Fig. 3c, Table S2). Shade-grown coffee varieties also formed distinct clusters, with *Ii*-G showing the highest rates and other associations like *Ea*-T, *Ha*-Oa, and *Ps*-Oa exhibiting the lowest ($F_{(6, 56)} = 17.98, p = 0.001; p < 0.033$). These differences highlight clear groupings in transpiration performance under different conditions (Fig. 4c, Table S2).

Stomatal conductance closely mirrored transpiration trends, showing a strong relationship between the two 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). In Oa cv, stomatal conductance varied significantly under unshaded conditions and shaded *Ha*-Oa and *Ps*-Oa associations ($F_{(3, 32)} = 8.697$, $p = 0.001$; $p < 0.033$) (Fig. 3d, Table S2). Shaded coffee varieties formed two distinct clusters, with *Iv*-Cr and *Ii*-G showing the highest conductance, while other combinations, including *Pg*-Ca, *Ip*-Oa, and *Ha*-Oa, exhibiting significantly lower values ($F_{(6, 56)} = 24.02$, $p = 0.001$; $p < 0.033$) (Fig. 4d, Table S2).

Ci values among shade trees showed variation, reflecting differences in carbon assimilation rates. The highest values were observed in *Iv*, *Ha*, and *Ii*, intermediate values in *Ps* and *Ip*, and lower values in *Pg* and *Ea*, with significant differences between clusters ($F_{(6, 56)} = 48.22$, $p = 0.001$; $p < 0.033$) (Fig. 2e, Table S1). For Oa, shaded and unshaded conditions influenced Ci values, with *Ip*-Oa showing the highest and *Ps*-Oa the lowest values ($F_{(6, 56)} = 48.22$, $p = 0.001$; $p < 0.033$) (Fig. 3e, Table S2). Among shaded varieties, one cluster, including *Ip*-Oa, *Iv*-Cr, and *Ii*-G, exhibited the highest Ci values, while another, consisting of *Ea*-T, *Pg*-Ca, *Ha*-Oa, and *Ps*-Oa, had lower values, with significant differences between the groups ($F_{(6, 56)} = 11.89$, $p = 0.001$; $p < 0.033$) (Fig. 4e, Table S2).

PAR values varied significantly among tree species. *Ea* showed the highest values, followed by an intermediate group including *Pg*, *Iv*, *Ha*, and *Ii*, while *Ip* and *Ps* had the lowest values. Significant differences were observed ($F_{(6, 56)} = 64.15$, $p = 0.001$; $p < 0.033$), particularly between *Ii* and some other species within the intermediate cluster (Fig. 2f, Table S1). For coffee plants, unshaded Oa had a significantly higher PAR compared to shaded conditions, with differences of 169-fold, 96.6-fold, and 53.7-fold compared with *Ps*-Oa, *Ha*-Oa, and *Ip*-Oa, respectively ($F_{(3, 32)} = 1159$, $p = 0.001$; $p < 0.033$) (Fig. 3f, Table S2). Among the shaded Arabica cvs, *Iv*-Cr and *Pg*-Ca had the highest PAR values. *Iv*-Cr PAR values were significantly higher than other associations, including *Pg*-Ca ($F_{(6, 56)} = 47.68$, $p = 0.001$; $p < 0.033$) (Fig. 4f, Table S2).

Instantaneous leaf water-use efficiency (ILWUE)

ILWUE values for the seven tree species varied from 0.83 ± 0.15 to 16.92 ± 11.05 µmol CO_2/mmol H_2O, values that correspond to *Ii* and *Ea*, respectively. Significant differences were observed between shade tree species ($F_{(6, 56)} = 5.810$; $p < 0.001$; $p < 0.033$), where the highest ILWUE were observed for *Ea* and *Ip*. In second place, a cluster formed by *Pg*, *Ha*, and *Ps* was observed with intermediate values. Also, a third cluster was observed, integrated by *Ii* and *Iv*, with the lowest ILWUE values observed (Fig. 5a, Table S1).

Concerning Oa cv, values varied from 4.61 ± 10.06 to 21.62 ± 11.27 µmol CO_2/mmol H_2O, values calculated for unshaded Oa and for the *Ha*-Oa shaded association. Unshaded bushes had lower ILWUE values compared with bushes under shaded conditions; this difference was statistically significant (F $_{(3, 32)}$ = 8.538; $p < 0.001$; $p < 0.033$), and at least 2-fold lower regarding shaded associations (Fig. 5b, Table S2). Finally, for shaded Arabica cvs, values varied from 7.12 ± 3.78 to 21.62 ± 11.27 µmol CO_2/mmol H_2O, calculated values for *Iv*-Cr for and *Ha*-Oa shaded associations, respectively. Significant differences were observed (F $_{(6, 56)}$ = 4.315; $p < 0.001$; $p < 0.033$) only for *Ha*-Oa, the association with the highest ILWUE values, regarding *Ii*-G and *Iv*-Cr, associations with the lowest values observed (Fig. 5c, Table S2).

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 also 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, photosynthetic efficiency, water-use efficiency, and resilience within coffee agroforestry systems for smallholders in Veracruz, Mexico. Our results demonstrate that the studied tree species contribute differently to the agroforestry system C capture. This, combined with non-stressful radiation levels, improved water-use efficiency in both the tree species and coffee varieties, as well as higher nitrogen and moisture content in shaded coffee plants compared to unshaded ones, supports our hypothesis. In addition, the findings highlight the diverse nature of agroforestry systems and the improved microclimatic conditions created by tree shade.

Dendrometric parameters of shade trees and carbon stock determination

Comparisons of our results with other studies can provide insights but are difficult due to variation in key variables. Tree density in coffee agroforestry systems is managed by producers who propagate preferred species in greenhouses or use seedlings from reforestation programs with native flora. In this sense, research on optimal tree densities is essential to minimise competition while enhancing coffee productivity. Beyond microclimatic differences across coffee-growing regions, factors like altitude, slope, and shade management significantly influence phenotypic traits, population densities, and ecosystem service synergies (Cerda et al., 2017; Asanok et al., 2024). For instance, higher altitudes reduce ambient temperatures, altering temperature-dependent phenological processes (Bewley and Black, 1994). Additionally, environmental factors beyond producer intervention also impact tree population densities. According to the available literature, the contribution to C capture of *I. inicuil*, a member of the Fabaceae family, contributed 33.77% of carbon uptake in a hectare containing 660 trees. This density is in the range reported in other studies, where all the individuals of three species of the *Inga* genus together with other economically important species can range from 100 to 800 trees ha^{-1}, with an average density of 250–350 trees ha^{-1} (Barradas and Fanjul, 1986; Soto-Pinto et al., 2001). Carbon storage in *I. inicuil* represents 28.3% and 47.17% of the total CS incorporated as

P. schiedeana and *E. americana* biomass, respectively, over the first ten years of growth, the highest levels in C capture observed in our study. The incorporation of *I. inicuil* into agroforestry systems can lead to 198 Mg C ha^{-1} of carbon sequestration, which is higher than coffee agroforestry systems with other *Inga*, *Erythrin*a, and other Musaceae species (91.64–115.5 Mg C ha^{-1}; Haber, 2001), increasing the agroforestry systems' effectiveness by incorporating *I. inicuil*. After 10 years of growth, *I. inicuil* exhibited a CS of 20.9 Mg C ha^{-1} for 200 trees ha^{-1}, exceeding values reported for *Inga densiflora* Benth. (24.3 Mg C ha^{-1} for 400 trees ha^{-1} over 20 years) with similar age and size parameters (Salazar, 1985; Kursten and Burschel, 1993). However, our values were three times lower than those reported for *I. inicuil* trees aged 10–30 years (64.3 Mg C ha^{-1}) with 164 trees ha^{-1} in Oaxaca, where regional factors influence tree density and size (Hernández-Vásquez et al., 2012; Alessandrini et al., 2011; Téllez et al., 2020). Although *I. punctata* had lower AGB and CS, its high density (20–40% of total trees) significantly contributed to carbon capture in the agroforestry system (Soto-Pinto et al., 2001). Including *I. punctata* and *I. vera* could achieve 91.64 Mg C ha^{-1} in forested regions with *Inga spp.* (Haber, 2001). Despite their role in carbon capture and promotion by government programs, *Inga spp.* do not appear to influence soil or crop nutrition (Romero-Alvarado et al., 2002). *E. americana* and *P. schiedeana*, with approximately 40 trees per hectare, showed AGB and CS per tree due to their larger trunk diameters and heights. The lower density of *E. americana* is attributed to grower preferences and ecophysiological requirements, such as seed dormancy that requires scarification for germination (Bewley and Black, 1994; Bonfil-Sanders et al., 2008). *P. schiedeana*, despite its high CS and large crown, has low density due to agroforestry configurations ensuring homogeneous shade in coffee plantations, as it can grow over 20 m tall (Niembro, 1992; Vázquez-Torres et al., 2017). Habitat loss also impacts *P. schiedeana* population densities (Cruz-Castillo et al., 2001; López et al., 1999).

For *H. appendiculatus*, previously reported densities range from 16–20% of tree strata in Chiapas coffee farms (Soto-Pinto et al., 2001; Castillo-Capitan et al., 2014). In our study, *H. appendiculatus* comprised 6% of 660 trees per hectare but could reach 11% at 350 trees per hectare, in line with densities reported by Soto-Pinto et al. (2001). Despite low density, *H. appendiculatus* significantly contributes to CS after 10 years. Its germination is inhibited by leaf litter due to an unknown dormancy mechanism, which could involve photoblastic germination or seed polymorphism (Vázquez-Yanes and Orozco-Segovia, 1992; Figueroa and Vázquez-Yanes, 2002). Producers might utilise this mechanism to enhance germination for reforestation and shade management.

As an exotic species, *P. guajava* represents 4–5% of tree density in coffee plantations (Soto-Pinto et al., 2001). Although our determined AGB and CS at 10 years are consistent with previous findings (Nava et al., 2009), the calculated biomass has among the lowest values of CS that we registered, linked to its average height of 3–8 meters (Hauzé et al., 2017). These characteristics could be used to add more trees of this species without affecting coffee production. Also, *P. schiedeana* 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).

The density per hectare reported in earlier studies involving the same species served as the basis for the classifications we employed in our investigation. In this context, tree density variations within coffee agroforestry systems influence resource availability, competition, and biomass production, which are critical for assessing their role in climate change mitigation and ecosystem services. Therefore, understanding these population densities across regions is essential to evaluate their functional traits and carbon sequestration potential accurately.

Agroforestry C capture supports smallholder conservation efforts while reducing the carbon footprint of industries and transport (Magnago et al., 2015; Lal et al., 2015; Zomer et al., 2017). These efforts could be translated into carbon offset markets, financial instruments designed to incentivise carbon emissions reduction, which could increase incomes for smallholders in retribution of their conservative efforts. Carbon offsets can benefit smallholders, including those managing less than two hectares, who often face barriers such as limited knowledge, lack of information, and high adoption costs (Feliciano et al., 2014; Buck and Palumbo-Compton, 2022). Addressing these challenges requires programs and policies that connect producers with compensation credit opportunities.

Photosynthetic and gas exchange properties of shade trees and coffee plants

The Fv/Fm values of six shade trees studied exceeded the 0.75 threshold (Fig. 2, Table S1), which is a boundary value for fully functional PSII (Lepeduš et al., 2005) and ranged from 0.76 to 0.81, while values for *E. americana* were below this threshold. Photosynthetic rates are influenced by light conditions, and higher rates suggest a greater physiological and biochemical capacity for carbon fixation (Gulmon and Chu 1981). Based on the evidence that atmospheric CO_2 concentrations can alter the quantum yield of non-cyclic electron transport, with both parameters showing a linear relationship (Genty et al., 1989), the lower Fv/Fm values observed in *E. americana* could be related to the lower intercellular CO_2 concentration observed.

Few studies have evaluated leaf gas exchange in the tree species analysed. For the *Persea* genus, including avocado species like *P. americana* Mill., CO_2 assimilation rates vary among cultivars, ranging from 8 to 12 μmol CO_2 m²/s (Yin et al., 2023). In another study, maximum assimilation reached 18.3 μmol CO2 m²/s in April (spring) and dropped to 10.2 μmol CO_2 m²/s in October (autumn) (Whiley, 1994). For *P. americana* variety Colin V-33, recent values averaged 1.16 μmol CO_2 m²/s, 0.79 mmol H_2O m²/s, 29.55 mmol m²/s, and 238.87 ppm for CO_2 assimilation, transpiration, stomatal conductance, and internal CO_2 concentration, respectively (Useche-Carrilo et al., 2022). While our measurements were lower overall, similar trends were observed, except for internal CO_2 concentration, which was 1.7 times lower. Variations in stomatal frequency among species, influenced by a variety of factors (Fricker and Willmer 2012), directly affects gas exchange profiles (Barrientos-Priego and Sánchez-Colín, 1987; Barrientos-Priego et al., 2003). In avocados, the system is also influenced by the leaf's location inside the canopy.

This is because light influences the stomata's rate of opening, where stomata formed in shade opening more slowly (Heath and Arpaia, 2004). Limited data on other *Persea* species highlights the need for research on native species, such as *P. schiedeana*, which offer benefits for coffee farms.

Concerning the *Inga* genus, in *Inga marginata* and *Inga subnuda*, maximum gross photosynthetic rates of 10.60 ± 1.42 and 11.65 ± 0.97 µmol CO_2 m²/s were recorded at light saturation points of approximately 365–379 µmol photons m²/s, chamber temperature of 27 °C, and CO_2 concentration of 394.92 ± 2.69 µmol mol^{-1} (dos Santos Pereira et al., 2019). In contrast, our study observed lower values of 1.67 ± 0.38, 2.21 ± 1.19, and 5.02 ± 1.34 µmol CO_2 m²/s for *I. inicuil*, *I. vera*, and *I. punctata*, respectively, under different conditions: 1000 µmol photons m²/s, 23.43 ± 0.37 °C, and fully developed leaves from 10–14-year-old trees near the understory, unlike the younger leaves of trees under controlled conditions analysed by dos Santos Pereira et al. (2019). These discrepancies may be attributed to factors such as leaf and plant age, light intensity, CO_2 concentration, and temperature (Bielczynski et al., 2017; Wohlfahrt, 2015). Additionally, diurnal patterns of photosynthesis and transpiration in coffee shade agroforestry systems revealed higher rates in sunlit leaves compared to those in shaded understory conditions with 40% irradiance (Siles et al., 2012).

For the *Erythrina* genus, variations in photorespiration and CO_2 absorption were observed. For *Erythrina poeppigiana* (Walp.) O. F. Cook, values ranged from 16.9 to 19.9 µmol CO_2 m²/s at 2000 µmol photon m²/s, 350 µmol/mol CO_2 concentration, and 28 °C, while 2-fold higher values occurred at 1000 µmol CO_2/mol (Nygren, 1995). Similarly, a CO_2 absorption rate of 15.49 µmol CO_2 m²/s was recorded for *Erythrina crista-galli* L. (Davis and Hidayati, 2019). The lower values that we observed are linked to factors like light intensity (Taiz et al., 2014), nitrogen availability for Rubisco regeneration (von Caemmerer and Farquhar, 1981; Cromer et al., 1993), stomatal conductance influenced by relative humidity (Taiz et al., 2014), the maximum amount of carbohydrates generated for each unit of transpired water (Cowan, 1977), ambient-to-intercellular CO_2 concentration ratios (Long, 1985), and mesophyll capacity for carbon fixation (Wong et al., 1979). This evidence highlights the role of stomatal conductance and chlorophyll content in CO_2 absorption, though further data is needed to discern species-specific patterns.

For *H. appendiculatus*, CO_2 assimilation rates of 3.7, 4.9, and 11.6 µmol CO_2 m²/s were recorded for low and high red/ far red-light ratios and high irradiance, mimicking a condition with no filtered light irradiance by the tree canopy (Tinoco-Ojanguren and Pearcy, 1995); our measured values are consistent with this evidence, indicating a non-stressing radiation condition. Additionally, *H. appendiculatus* has been described as a primary succession pioneer species with a preference for gaps where light is readily available, enabling it to assimilate this high radiation into higher carbon gain (Stegemann et al., 1996). These studies show that the quality of light filtering down to the shrub layer induces changes in *H. appendiculatus* CO_2 assimilation behaviour, adapting their photosynthesis to the light conditions of defined niches.

In *P. guajava*, maximum CO_2 assimilation rates of 16–18 µmol CO_2 m²/s were measured between 11:00–14:00 h, dropping to near zero by 19:30 h (Nava et al., 2009). On the other hand, shaded and unshaded conditions yielded rates of 15.47 and 11.96 µmol CO_2 m²/s, respectively (Idris et al., 2019), highlighting reduced stomatal and gas exchange activity under lower light. Altitude and light intensity influence stomatal density, with density increasing under higher light (Shirke and Pathre, 2003; Casson and Gray, 2008). Our, observed rates of 9.18 µmol CO_2 m²/s align with these findings.

So far, some general assumptions can be made considering the species belonging to different families and analysed separately into three sections: *a) Carbon capture*, b*) Adaptation to climate change*, and *c) Productive diversification as a strategy to face climate change in coffee plantations*.

a) Carbon capture:

Species of the *Inga* genus (Fabaceae) exhibited a reduced net photosynthetic rate, smaller stomatal opening, and lower transpiration under an average temperature of 23.43 °C and 70.4% RH. Under lower temperatures and higher RH, increased CO_2 production from photorespiration and/or mitochondrial respiration reduces stomatal opening, which limits transpiration more significantly than photosynthesis (Shimshi and Ephrat, 1975). This physiological response gives CO_2 available to incorporate into biomass.

E. americana (Fabaceae) exhibited higher CO_2 fixation rates despite reduced stomatal opening and transpiration, accompanied by increased intercellular CO_2. This response, observed under higher RH and lower temperatures, enhances leaf water potential and CO_2 assimilation due to increased stomatal aperture at low VPD (Running, 1976; Dai, 2013; Grossiord et al., 2020). In contrast, *Inga* species showed slower stomatal closure at elevated temperatures, allowing transpiration to persist under similar conditions (Engineer et al., 2016; Xu et al., 2016). Species-specific variations in the relationship between stomatal conductance and CO_2 assimilation (Gil et al., 2009) reveal distinct capacities for carbon storage under environmental stress.

H. appendiculatus (Malvaceae) shows increased stomatal conductance and CO_2 fixation, resulting in higher intercellular CO_2. The stomatal conductance, which determines the rate of supply of CO_2 to the leaf interior, directly affects the value of Ci; therefore, higher conductance implies more readily available CO_2 in the leaf's intercellular spaces (Farquhar and Sharkey, 1982; Taiz et al., 2014). Also, its adaptations, such as increased leaf area and stomatal density under dim light, enhance CO_2 assimilation in fluctuating light conditions (Fetcher et al., 1983; Tinoco-Ojanguren and Pearcy, 1995; Friend, 1984); while, under high radiation environments, such as gaps, this specie tends to have higher carbon gain (Stegemann et al., 1996). On the other hand, *P. guajava* (Myrtaceae) shows a diurnal pattern where CO_2 assimilation increases early in the day but decreases later, with stomatal conductance nearing zero by evening (Nava et al., 2009). This pattern demonstrates efficient use of CO_2 during peak photosynthesis hours as have been seen in other species (Schussler and Westgate, 1988; Lima and Pinheiro, 2004; Romero et al., 2004).

No information is currently available regarding *P. schiedeana* (Lauraceae); however, insights can be drawn from studies on other taxa of the same genus. In *P. schiedeana*, low stomatal conductance and CO_2 fixation rates were observed, leading to decreased transpiration under high

RH (70.4%) and moderate temperatures (23.43°C). These patterns align with findings in *P. americana* Colin V-33 variety, where limited CO_2 assimilation correlated with high intercellular CO_2 (Ci) (Useche-Carrillo et al., 2022) due to metabolic saturation (Azcón-Bieto et al., 2008). Saturation occurs when the plant's metabolic machinery is saturated, leading to stomata closure (Sánchez-Díaz and Aguirreolea 2008; Pritchard and Amthor 2005; Fricker and Willmer 2012; Useche-Carrillo et al., 2022), halting photosynthesis, and blocking the processing of additional molecules, therefore causing an increase in CO_2 concentration until it reaches the saturation level, following typical saturation kinetics regarding its photosynthetic mechanism (C_3 or C_4) (Azcón-Bieto et al., 2008).

Arabica coffee plants displayed low CO_2 fixation rates in unshaded conditions due to stress from high photosynthetically active radiation and increased temperature (27.53°C versus 23.43°C in shaded areas). These differences suggest that unshaded coffee plants experience reduced carbon storage efficiency, as high temperatures hinder photosynthetic electron transport and increase photorespiration, since coffee plants are sensitive to temperature increases above 25 °C (Farquhar and Sharkey, 1982). This physiological response is mainly associated with the type of coffee photosynthesis (C_3 photosynthetic metabolism) (Mosquera-Sanchez et al., 1999).

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).

b) Adaptation to climate change:

Under high VPD, the *Inga* species studied experience reduced transpiration rates and photosynthetic activity as stomata close to minimise water loss. This pattern aligns with findings that high vapor pressure deficit (VPD) reduces transpiration in many species by triggering to guard cells lose turgor under high evaporation conditions, as the low water input is insufficient to satisfy the high transpiration rate (Gholipoor et al., 2010; Zaman-Allah, et al., 2011; Schoppach and Sadok, 2012; Jiao et al., 2019). As a result, the rate of photosynthetic activity declines when stomata close at a high VPD (Sinclair, 2017). Such responses are particularly relevant for species in arid climates or areas with fluctuating humidity levels, highlighting stomatal regulation as a critical adaptation mechanism.

E. americana, a pioneer species adapted to optimal temperatures near 28 °C (García-Mateos et al., 2001; Palma-Garcia and Gonzales-Rebeles Islas, 2018), reduces transpiration and increases stomatal aperture to regulate leaf water potential (Running, 1976; Dai, 2013; Grossiord et al., 2020) in order to maintain efficient water-use under stressful conditions, enhancing its capacity to withstand climate extremes. This result is in line with our ILWUE analysis, which shows that *E. americana* and *I. punctata* tend to decrease their transpiration rate in order to make efficient use of their water resources. This trait enhances the potential of the agroforestry system to withstand the effects of climate change.

H. appendiculatus, adapted to high-radiation environments, maintains robust photosynthetic performance in both intense and dim light by adjusting leaf traits such as thickness and stomatal density (Fetcher et al., 1983; Friend, 1984; Stegemann et al., 1996; Fahl et al., 1994), making this species more resilient to fluctuating light conditions, since light quality seems does not affect photosynthesis modulation in this species (Tinoco-Ojanguren and Pearcy, 1995), and enhancing its potential to efficiently assimilate the available CO_2. In contrast, *P. guajava* reduced CO_2 absorption under stress may relate to slower growth and vulnerability to drought (Maxwell and Johnson, 2000; Simonin et al., 2012; North et al., 2013).

Reduced CO_2 assimilation in *P. schiedeana* could also be linked to increased VPD, as elevated VPD reduces stomatal conductance, limiting CO_2 uptake (Schultze, 1986). This inverse relationship between stomatal conductance and VPD was similarly observed in *P. americana* under comparable conditions (Bower et al., 1978), indicating a shared adaptive mechanism to minimise water loss while coping with environmental stress. However, the accompanying reduction in photosynthetic activity limits its adaptation potential to extreme conditions, making it more vulnerable to fluctuating VPD levels.

Since coffee plants are undergrowth plants, a high PAR indicates that bushes in full sun are under stress; therefore, producers have to apply much more fertiliser, in addition to the fact that coffee plants under these conditions are more vulnerable to climate change variations. Arabica coffee plants displayed normal photosynthetic performance at the PSII level (Fv/Fm) under both shaded and unshaded conditions, despite significant variations in radiation (30–169 times higher in unshaded areas) and different management between agroforestry systems. However, in line with our observations, the detrimental effect of temperatures above 25 °C on stomatal and CO_2 assimilation conductance are more pronounced in unshaded plants, which are exposed to higher radiation and VPD, causing guard cell turgor loss and stomatal resistance (Makino et al., 1994; Nutman et al., 1937; Riaño 1993; Larcher, 1994; Roháček, 2002). Despite these challenges, shaded plants exhibit more stable CO_2 fixation rates due to moderated environmental conditions.

c) Productive diversification as a strategy to face climate change in coffee plantations:

By understanding the physiological responses of *Inga* genus and similar genera, strategies like intercropping or shading in coffee plantations can be optimised. Maintaining favourable microclimatic conditions to optimise plant physiological responses to extreme conditions may simultaneously enhance carbon storage and productivity, contributing to sustainable diversification under climate change scenarios.

The contrasting responses of *H. appendiculatus* and *P. guajava* highlight strategies to optimize CO_2 assimilation. While *H. appendiculatus* thrives in shade and fluctuating light, *P. guajava*'s efficient midday photosynthesis makes it suitable for agroforestry systems with controlled light conditions. These physiological traits can support productive diversification in coffee plantations, enhancing resilience to climate variability.

The physiological traits of *P. schiedeana*, such as low CO_2 assimilation and limited stomatal conductance, make it less competitive under high VPD conditions. However, its ability to function efficiently in moderate environments with high RH could support productive diversification when integrated into agroforestry systems. Proper management of microclimates may optimise its photosynthetic potential and enhance its role in sustainable coffee plantation systems.

The stress tolerance observed in shaded coffee plants underscores the value of agroforestry systems in mitigating the effects of climate change. Shade-grown conditions reduce dependency on fertilizers and enhance photosynthetic efficiency, making them a viable strategy for sustainable coffee production. The physiological resilience of certain coffee varieties under shaded conditions suggests that integrating agroforestry into plantation management can help maintain productivity while protecting plants from the adverse effects of extreme environmental conditions.

Instantaneous leaf water-use efficiency (ILWUE)

According with our results, *I. punctata* and *E. americana* exhibited the highest ILWUE values, indicating their potential to thrive under water-scarce conditions or other environmental stresses like drought, salinity, or extreme temperatures (Beer et al., 1997; Chaves et al., 2004; Sinclair, 2017). The ILWUE of the other five species showed no significant differences. Although prior ILWUE data for the studied tree species, their genera, or other montane cloud forest species were unavailable to compare our calculated values, these were generally higher or comparable to those of eight non-native tree species from urban ecosystems in Los Angeles, USA (McCarthy et al., 2011), except for *I. inicuil* and *I. vera*, which displayed the lowest ILWUE. Among the species studied by McCarthy et al. (2011) *Brachychiton discolour* F.Muell., *Brachychiton populneus* (Schott & Endl.) R.Br., and *Eucalyptus grandis* W.Hill ex Maiden, achieved the highest ILWUE and tree-level seasonal WUE (ratio between stem basal-area increment (BAI)/total transpiration Although a lower water use was not directly related to a low BAI, the species that used the least amount of water were those that are evergreen or come from areas with high VPD.

In a subtropical evergreen forest in Wuyi Mountain, China, stable carbon isotopic labeling revealed ILWUE values (11–40 µmol CO2/mmol H_2O) for five tree species (*Castanopsis eyrie* Champ. ex Benth.) Hutch., *Symplocos laurina* (Retz.) Noot., *Machilus grijsii* Hance, *Ternstroemia gymnanthera* (Wight & Arn.) Sprague, and *Rhododendron ovatum* (Lindl.) Planch. ex Maxim.) (Huang et al., 2023), with the highest value surpassing those calculated in our study for *E. americana*. Remarkably, they observed that ILWUE increased with altitude due to greater light exposure and water deficit, underscoring the role of habitat features like soil moisture, light intensity, and nutrient availability in influencing WUE. Their findings highlighted how changes in habitat features, particularly soil water availability, and light intensity, have a significantly influence on water use and nutrient status.

Concerning coffee bushes, ILWUE was assessed in 14 *C. arabica* cultivars during drought-tolerance selection. Eleven cultivars showed increased ILWUE during an 18-day drought, with values ranging from 5–7 µmol CO_2/mmol H_2O at 14 days to 4–12.5 µmol CO_2/mmol H_2O at 18 days (Reis et al., 2022). In contrast, ILWUE for five *C. arabica* cultivars in our study ranged from 4.6 to 21.62 µmol CO_2/mmol H_2O, with the shaded *H. appendiculatus*-Oro Azteca variety showing a two-fold higher ILWUE compared to drought-tolerant genotypes. Traits such as thicker cuticles, fewer xylem vessels, thicker phloem, higher stomatal density, and longer roots significantly enhanced water use efficiency (Baliza et al., 2012; Wolfe et al., 2016).

Also, seasonal gas exchange in 21 *C. arabica* genotypes revealed ILWUE values between 1.5–3.3 µmol CO_2/mmol H_2O in the rainy season and 1.2–3.4 µmol CO_2/mmol H_2O in the dry season (Tereza et al., 2022). These values were generally lower than those we calculated for the studied cultivars, except for the Oro Azteca cultivar in unshaded conditions. While photosynthetic rate and stomatal conductance declined in the dry season, ILWUE remained stable due to coordinated physiological adjustments. The observed changes can be explained through the genetic variability of the *C. arabica* genotypes; while the changes observed with respect to the change of season suggest a differential response of each genotype to drought.

Evidence from sorghum under water-limited conditions (Warrington and Kanemasu, 1983) and maize in agroforestry systems with *Grevillea robusta* A. Cunn. (Lott, 1998) shows that above the light saturation point, increasing radiation reduces instantaneous water use efficiency. A similar pattern may apply to *C. arabica* varieties, as the shaded Oro Azteca variety showed higher WUE compared to unshaded conditions (higher PAR conditions), combined with increased transpiration and a reduced water efficiency. Despite its resilience to unshaded conditions, Oro Azteca variety exhibited reduced photosynthetic rates and increased water loss under these conditions. Variations among shaded cultivars could be attributed to genotypic, phenotypic, and environmental factors (Medrano et al., 2007). According to this, ILWUE is influenced by different factors, which are related to the inherent characteristics of the plant species and variety, particularly their ability to optimise resource use for growth and survival (Kirkham, 2005).

ILWUE reflects plant resource optimization for growth and survival, focusing on the interactions between CO_2, water availability, and temperature (Hatfield and Dold, 2019). However, ILWUE's limitation lies in its short-term measurement, complicating its extrapolation to whole-plant water efficiency (Medrano et al., 2007). Understanding WUE in native species is critical for reforestation and conservation in water-scarce regions.

Understanding the water-use efficiency of native species is vital for reforestation and conservation in water-scarce regions. Native species, adapted to local conditions, maximize biomass growth and carbon sequestration while minimising water use (Hatfield and Dold, 2019). Similarly, incorporating high-WUE native trees in coffee agroforestry systems can enhance sustainability, productivity, and resilience against climatic variability, aligning ecological and agricultural goals.

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 number of farms, measured leaves, and environmental conditions. Addressing these requires longitudinal studies across seasons, the performing of curves to different light intensities, temperatures, and environmental CO_2 concentrations during different seasons of the year under unshaded and shaded conditions. Likewise, the inclusion and development of permanent sample plots should be considered, as well as the implementation of a robust experimental design that helps to rule out the different biases during the measurement of the selected individuals.

Conclusions

This work counts to increase knowledge about the native species that compose coffee agroforestry systems, being a support for their selection through their carbon capture contribution and physiological characteristics. Species such as *I. inicuil*, *P. schiedeana*, and *E. americana* were identified as particularly valuable. *I. inicuil* contributes the most to carbon sequestration due to its high density; while *E. americana* and *P. schiedeana* contributed significantly to carbon capture due to their large sizes and high growth rates. *E. americana* offers additional benefits such as, nitrogen fixation, high-nutritional leaf litter for small ruminants, and valuable wood for handicrafts, while increasing the population density of *Ps* could further enhance its shade benefits and the economic potential of its fruit. Additionally, our results revealed that *E. americana* and *I. punctata* achieving the highest WUE, emphasising the potential of high-WUE species to enhance agroforestry systems' sustainability, especially in regions with limited water resources. We suggest that these tree species have the physiological behaviour most suitable for the type of agroforestry system under investigation, contributing differentially to climatic resilience due to their inherent genotypic and physiological characteristics. Also, species such as *P. guajava* and *H. appendiculatus* adapt well to coffee plantations due to their high photosynthetic efficiency and effective CO_2 usage, making more efficient use of the available resources. These findings highlight the differential physiological behaviours of species and their roles in enhancing agroforestry sustainability and climatic resilience. It is important to note that selection must include the rest of the species that make up the coffee farms in order to not promote monospecific shade in the crops; therefore, further studies are needed on the carbon capture capacity, physiological status, and climatic resilience of the species.

Due to the enhanced microclimatic conditions provided by shade trees, coffee plants and all tree species evaluated here experienced stress-free conditions at the photosynthetic level, while leaf gas exchange and stomatal function are significantly influenced by RH, specifically the VPD. Shaded coffee cultivars, particularly *Ha*-Oa, exhibited superior ILWUE compared to unshaded ones, highlighting the benefits of shading for water use optimisation. Shade not only reduced photosynthetic stress but also increased nitrogen and moisture content in coffee plants, enhancing carbon sequestration and photoprotective capacity; while the observed variations in

CO_2 fixation and sugar concentrations between individuals and varieties further underscored heterogeneity between individuals and varieties.

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 prospective 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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References

Acevedo G, Beinroth FH, Dubee BC, Esnard AM, Hernandez PJ, Liegel LH, Lugo-Lopez MA, 1992. Organic Carbon Sequestration in the Soils of Puerto Rico: a Case Study of a Tropical Environment. Dayton Publishing Services, Mayagüez, Puerto Rico.

Adugna D, Struik P. 2011. Effects of shade on growth, production and quality of coffee (*Coffea arabica*) in Ethiopia. *Journal of Horticulture and Forestry* 3(11):336-341.

Akter R, Hasan M K, Kabir K H, Darr D, Roshni NA. 2022. Agroforestry systems and their impact on livelihood improvement of tribal farmers in a tropical moist deciduous forest in Bangladesh. *Trees, Forests and People*, 10:100315. https://doi.org/10.1016/j.tfp.2022.100315

Alessandrini A, Biondi F, Di Filippo A, Ziaco E, Piovesan G. 2011. Tree size distribution at increasing spatial scales converges to the rotated sigmoid curve in two old-growth beech stands of the Italian Apennines. *Forest Ecology and Management* 262(11), 1950–1962. https://doi.org/10.1016/10.1016/j.foreco.2011.08.025

Araujo WL, Dias PC, Moraes GA, Celin EF, Cunha RL, Barros RS, DaMatta FM. 2008. Limitations to photosynthesis in coffee leaves from different canopy positions. *Plant Physiology and Biochemistry* 46(10):884–890. https://doi.org/10.1016/j.plaphy.2008.05.005

Araza A, de Bruin S, Lucas R, et al. 2022. A comprehensive framework for assessing the accuracy and uncertainty of global above-ground biomass maps. *Remote Sensing of Environment*, 272:112917. https://doi.org/10.1016/j.rse.2022.112917

Asanok L, Krueama K, Pakketanang J, et al. 2024. Variation of shade tree composition and carbon stock of smallholder coffee agroforestry systems along an elevation gradient in Khun Mae Kuang Forest area, northern Thailand. *Agroforest Systems*. https://doi.org/10.1007/s10457-024-01073-9

Azcón-Bieto J, Fleck I, Aranda X, Gómez-Casanovas N. 2008. Transpiración y control estomático. In Azcón-Bieto, J, Talón M (Eds) Fundamentos de Fisiología Vegetal. McGraw-Hill Interamericana, España, pp. 247-263.

Baffes J, Lewin B, Varangis P. 2005. Coffee: Market setting and policies. In: Ataman M, Beghin J, eds. Global Agricultural Trade and Developing Countries. World Bank, Washington, D.C.

Baker NR, Rosenqvist E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany*, 55(403):1607-1621. https://doi.org/10.1093/jxb/erh196

Baker NR. 2008. Chlorophyll Fluorescence: A Probe of Photosynthesis *In Vivo*. *Annual Review of Plant Biology* 59(1):89-113. https://doi.org/10.1146/annurev.arplant.59

Baliza DP, Cunha RL, Guimarães RJ, Barbosa JPRAD, Ávila FW, Passos AMA. 2012. Physiological characteristics and development of coffee plants under different shading levels. *Revista Brasileirade Ciencias Agrarias* 7(1):37-43. https://doi.org/10.5039/agraria.v7i1a1305

Barradas VL, Fanjul L. 1986. Microclimatic characterization of shaded and open-grown coffee (*Coffea arabica* L.) plantations in Mexico. *Agricultural and Forest Meteorology* 38(1-3):101-112. https://doi.org/10.1016/0168-1923(86)90052-3

Barrientos-Priego A, López-Jiménez A, Sánchez-Colín S. 1987. Effect of Colín V-33 as interstock on avocado (*Persea americana* Mill.) growth, cv. Fuerte. South African Avocado Growers Association Yearbook, 10:62-63.

Barrientos-Priego AF, Borys MW, Trejo C, López- López L. 2003. Índice y densidad estomática foliar en plántulas de tres razas de aguacatero. *Revista Fitotecnia Mexicana* 26(4):285-290

Beer J, Muschler R, Kass D, Somarriba E. 1998. Shade management in coffee and cacao plantations. *Forestry Sciences* 38:139-164. https://doi.org/10.1007/978-94-015-9008-2_6

Bewley J, Black M. 1994. Seeds: physiology of development and germination, 2nd Edn. Plenum Press, New York.

Bhattacharya A. 2019. Water-Use Efficiency Under Changing Climatic Conditions, Academic Press, 111–180. https://doi.org/10.1016/b978-0-12-816209-5.00003-9

Bielczynski LW, Łącki MK, Hoefnagels I, Gambin A, Croce R. 2017. Leaf and Plant Age Affects Photosynthetic Performance and Photoprotective Capacity. *Plant Physiology* 175(4):1634-1648. https://doi.org/10.1104/pp.17.00904

Bilen C, El Chami D, Mereu V, Trabucco A, Marras S, Spano D. 2023. A Systematic Review on the Impacts of Climate Change on Coffee Agrosystems. *Plants* 12(1):102. https://doi.org/10.3390/plants12010102

Bizualem A, Degye G, Zekarias S. 2015. Analysis of marketed surplus of coffee by smallholder farmers in Jimma zone, Ethiopia. *Journal of Biology, Agriculture and Healthcare* 5(5).

Bonfil-Sanders C, Cajero-Lázaro I, Evans R. 2008. Germinación de semillas de seis especies de *Bursera* del centro de México. *Agrociencia* 42(7):827-834.

Bower JP, Wolstenholme BN, de Jager JM. 1978. Incoming solar radiation and internal water status as stress factors in avocado, *Persea americana* Mill. cv. Edranol. *Crop Production* 7:129-33.

Buck HJ, Palumbo-Compton A. 2022. Soil carbon sequestration as a climate strategy: what do farmers think? *Biogeochemistry* 161:59-70. https://doi.org/10.1007/s10533-022-00948-2

Bunn, C., Läderach, P., Ovalle Rivera, O. et al. 2015 A bitter cup: climate change profile of global production of Arabica and Robusta coffee. Climatic Change 129, 89-101. https://doi.org/10.1007/s10584-014-1306-x

Casson S, Gray JE. 2008. Influence of environmental factors on stomatal development. *New Phytologist* 178:9-23. https://doi.org/10.1111/j.1469-8137.2007.02351.x

Castillo-Capitán G, Ávila-Bello CH, López-Mata L, de León González F. 2014. Structure and tree diversity in traditional popoluca coffee agroecosystems in the Los Tuxtlas Biosphere Reserve, Mexico. *Interciencia* 39(9):608-619.

Cerda R, Allinne C, Gary C, Tixier P, Harvey CA, Krolczyk L, Avelino J. et al. 2017. Effects of shade, altitude and management on multiple ecosystem services in coffee agroecosystems. *European Journal of Agronomy*, 82:308-319. https://doi.org/10.1016/j.eja.2016.09.019

Chave J, Réjou-Méchain M, Búrquez A, Vieilledent G, et al. 2014. Improved allometric models to estimate the aboveground biomass of tropical trees. *Global Change Biology* 20(10):3177-3190. https://doi.org/10.1111/gcb.12629

Chaves MM, Osório J, Pereira JS. 2004. Water use efficiency and photosynthesis. In Bacon MA (Ed) Water use efficiency in plant biology, 42–74. Blackwell Publishing, Oxford, UK.

Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). 2023. Enciclovida. https://enciclovida.mx. Accessed on 10 January 2023

Cowan IR. 1977. Stomatal behavior and environment. *Advances in Botanical Research* 4:117-227. https://doi.org/10.1016/S0065-2296(08)60370-5

Cromer RN, Kriedemann PE, Sands PJ, Stewart LG. 1993. Leaf growth and photosynthetic response to nitrogen and phosphorus in seedling trees of *Gmelina arborea*. *Australian Journal of Plant Physiology* 20:83-98. https://doi.org/10.1071/PP9930083

Cruz-Castillo JG, Torres-Lima PA, Rodríguez-Bracamontes F, Martínez-Pérez D. 2001. Adaptación de frutales caducifolios. Revisión comparativa de Guatemala y Veracruz, México. *Sociedad Rurales, Producción y Medio Ambiente* 2:63-73.

Dai AG. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change* 3:52-58. https://doi.org/10.1038/nclimate1633

Dávalos-Sotelo R, Morato IM, Martínez-Pinillos-Cueto E. 2008. Almacenamiento de carbono. In: Manson RH, Hernández-OrtízV, Gallina S, Mehltreter K (Eds) Agroecosistemas cafetaleros de Veracruz biodiversidad, manejo y conservación. Instituto de Ecología, Instituto Nacional de Ecología. México. pp: 223-233.

Davidson S. 2004. Shade coffee agro-ecosystems in Mexico. *Journal of Sustainable Forestry* 21(1):81-95. https://doi.org/10.1300/J091v21n01_05

Davis L, Hidayati N. 2019. Carbon dioxide absorption and physiological characteristics of selected tropical lowland tree species for revegetation. Conference Series: *Earth and Environmental Science, Tangerang, Indonesia* 591:012039. https://doi.org/10.1088/1755-1315/591/1/012039

Diario Oficial de la Federación (DOF). 2019. NMX-Y-359-SCFI-2019: Alimentos para animales-determinación de proteína cruda método de Kjeldahl en alimentos balanceados e ingredientes mayores official method 984.13, A-D.

Donald PF. 2004. Biodiversity Impacts of Some Agricultural Commodity Production Systems. *Conservation Biology* 18:17-38. https://doi.org/10.1111/j.1523-1739.2004.01803.x

dos Santos Pereira HA, da Costa GS, Dalmolin ÂC, et al. 2019. Photosynthesis, growth, and biomass allocation responses of two Inga species to contrasting light. *Acta Physiologiae Plantarum* 41:174. https://doi.org/10.1007/s11738-019-2966-y

Ehrenbergerová L, Šenfeldr M, Habrova H. 2018. Impact of tree shading on the microclimate of a coffee plantation: A case study from the Peruvian Amazon. *Bois et Forest des Tropiques* 334(4):13. https://doi.org/10.19182/bft2017.334.a31488

Ellis EA, Baerenklau KA, Marcos-Martínez R et al (2010) Land use/land cover change dynamics and drivers in a low-grade marginal coffee growing region of Veracruz, Mexico. *Agroforestry Systems* 80:61–84. https://doi.org/10.1007/s10457-010-9339-2

Engineer CB, Hashimoto-Sugimoto M, Schroeder JI, et al. 2016. CO_2 Sensing and CO_2 Regulation of Stomatal Conductance: Advances and Open Questions. *Trends in Plant Science* 21(1):16-30. https://doi.org/10.1016/j.tplants.2015.08

Estrada-Contreras I, Equihua M, Castillo-Campos G, Rojas-Soto O. 2015. Climate change and effects on vegetation in Veracruz, Mexico: an approach using ecological niche modelling. *Acta botánica mexicana* 112:73-93. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-71512015000300007&lng=es&tlng=en.

Fahl JI, Carelli MC, Vega J, Magalhaes AC. 1994. Nitrogen and irradiance levels affecting net photosynthesis and growth of young coffee plants. *Coffea arabica* L.). *Journal of Horticultural Science* 69:161-169. https://doi.org/10.1080/14620316.1994.11515262

Fairtrade Foundation. 2022. Coffee farmers. Fairtrade Foundation. Available online: https://www.fairtrade.org.uk/farmers-and-workers/ coffee/

Farquhar GD, Sharkey TD. 1982. Stomatal Conductance and Photosynthesis. *Annual Review of Plant Physiology* 33(1):317-345. https://doi.org/10.1146/annurev.pp.33.0601

Feliciano D, Hunter C, Slee B, Smith P. 2014. Climate change mitigation options in the rural land use sector: Stakeholders' perspectives on barriers, enablers and the role of policy in North East Scotland. *Environmental Science & Policy* 44: 26-38. https://doi.org/10.1016/j.envsci.2014.07.0

Fetcher N, Strain BR, Oberbauer SF. 1983. Effects of light regime on the growth, leaf morphology, and water relations of seedlings of two species of tropical trees. *Oecologia* 58(3):314-319. https://doi.org/10.1007/bf00385229

Figueroa JA, Vázquez-Yanes C. 2002. Efecto de la calidad de la luz sobre la germinación de semillas en el árbol pionero tropical *Heliocarpus appendiculatus*. Tiliaceae). *Revista de Biología Tropical* 50(1):31-6

Fini A, Vigevani I, Corsini D, Wężyk P, Viskanic P, et al. 2023. CO_2-assimilation, sequestration, and storage by urban woody species growing in parks and along streets in two climatic zones. *Sci Total Environ*. 903:166198. https://doi.org/10.1016/j.scitotenv.2023.166198.

Food and Agriculture Organization of the United Nations (FAO). 2022. Markets and trade: Coffee. FAO. https://www.fao.org/markets-and-trade/commodities/coffee/en/

Fricker M, Willmer C. 2012. Stomata. Springer Science & Business Media, Netherlands.

Friend DJC. 1984. Shade adaptation of photosynthesis in *Coffea arabica*. *Photosynthesis Research* 5(4):325-334. https://doi.org/10.1007/BF00034977

García-López T. 2009. La política mexicana de acción climática y su aplicación al estado de Veracruz. *Revista de Investigaciones Políticas y Sociológicas* 8:153-167

García-Mateos R, Soto-Hernández M, Vibrans H. 2001. *Erythrina Americana* Miller. "Colorín"; Fabaceae), a versatile resource from Mexico: A Review. *Economic Botany* 55:391-400. https://doi.org/10.1007/BF02866562

Garza-Lau R, Maldonado-Torres R, Álvarez-Sánchez M E, Buendía-Espinoza J C. 2020. Aporte nutrimental de especies arbóreas fijadoras de nitrógeno en sistemas agroforestales con café. *Revista Mexicana De Ciencias Agrícolas* 11(4):801-814.

Genty B, Briantais JM, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA) - General Subjects* 990(1):87-92. https://doi.org/10.1016/s0304-4165(89)80016-9

Gholipoor M, Prasad PVV, Mutava RN, Sinclair TR. 2010. Genetic variability of transpiration response to vapor pressure deficit among sorghum genotypes. *Field Crops Research* 119(1), 85-90. https://doi.org/10.1016/j.fcr.2010.06.018

Gil PM, Gurovich L, Schaffer B, García N, Iturriaga R. 2009. Electrical signaling, stomatal conductance, ABA and ethylene content in avocado trees in response to root hypoxia. *Plant Signaling & Behavior* 4(2):100-108. https://doi.org/ 10.4161/psb.4.2.7872

Gill AS, Prasad JVNS. (2000). Allelopathic interactions in agroforestry systems. In: Narwal, S.S., Hoagland, RE, Dilday RH, Reigosa MJ (Eds) Allelopathy in Ecological Agriculture and Forestry. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-4173-4_13

Gliessman S. 2015. Agroecology: the Ecology of Sustainable Food Systems. 3rd Edition. Boca Raton, Florida: CRC Press/Taylor and Francis. p. 406. https://doi.org/ 10.1201/b17881

Gross, C.D., Bork, E.W., Carlyle, C.N., Chang, S.X., 2022. Agroforestry perennials reduce nitrous oxide emissions and their live and dead trees increase ecosystem carbon storage. *Glob. Change Biol.* 28:5956–5972. https://doi.org/10.1111/GCB.16322.

Grossiord C, Buckley TN, Cernusak LA, Novick KA, Poulter B, Siegwolf RTW, Sperry JS, McDowell NG. 2020. Plant responses to rising vapor pressure deficit. *New Phytologist* 226:1550-1566. https://doi.org/10.1111/nph.16485

Gulmon SL, Chu CC. 1981. The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparral shrub, *Diplacus aurantiacus*. *Oecologia* 49:207-212. https://doi.org/10.1007/BF00349189.

Guzmán Luna A, Bacon CM, Méndez VE, Flores Gómez ME, Anderzén J, Benavides González ÁN, et al. (2022) Toward Food Sovereignty: Transformative Agroecology and Participatory Action Research With Coffee Smallholder Cooperatives in Mexico and Nicaragua. *Front. Sustain. Food Syst.* 6:810840. https://doi.org/10.3389/fsufs.2022.810840

Haber J. 2001. International MBA. Informe de la Finca Santa Elena, productora de café orgánico. Tapachula, Chiapas, México. Available online: http://www.cca.cec.org/ files/pdf/ECONOMY/santa-elena-S.PDF.

Häger A, 2012. The effects of management and plant diversity on carbon storage in coffee agroforestry systems in Costa Rica. *Agrofor. Syst*. 86, 159–174. https://doi. org/10.1007/s10457-012-9545-1.

Harvey CA, Pritts AA, Zwetsloot MJ. et al. 2021. Transformation of coffee-growing landscapes across Latin America. A review. *Agronomy for Sustainable Development* 41:62. https://doi.org/10.1007/s13593-021-00712-0

Hatfield JL, Dold C. 2019. Water-Use Efficiency: Advances and Challenges in a Changing Climate. *Frontiers in Plant Science*, 10. https://doi.org/10.3389/fpls.2019.00103

Hausermann H. 2014. Maintaining the coffee canopy: understanding change and continuity in Central Veracruz. *Hum Ecol* 42:381– 394. https://doi.org/10.1007/s10745-014-9644-x

Heath R, Arpaia M. 2004. Avocado tree physiology - understanding the basis of productivity. Proceedings of the California Avocado Research Symposium 65-88.

Hernández-Vásquez E, Campos-Ángeles GV, Enríquez del Valle JR, Rodríguez-Ortiz G, Velasco-Velasco VA. 2012. Captura de carbono por *Inga jinicuil* Schltdl: En un sistema agroforestal de café bajo sombra. *Revista mexicana de ciencias forestales* 3(9):11-21. Available online: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-11322012000100002&lng=es&tlng=.

Heuzé V, Tran G, Bastianelli D, Lebas F. 2017. Guava (*Psidium guajava*) Feedipedia. https://agritrop.cirad.fr/582480/7/ID582480_ENG.pdf. Accessed 03 August 2023.

Huang KX, Xue ZJ, Wu JC, Shi FX, et al. 2023. Water Use Efficiency of Five Tree Species and Its Relationships with Leaf Nutrients in a Subtropical Broad-Leaf Evergreen Forest of Southern China. *Forests* 14(12):2298. https://doi.org/10.3390/f14122298

Idris A, Linatoc AC, Bin Abu Bakar MF, Takai ZI. 2019. Effect of light intensity on the gas exchange characteristics and total pigment content of *Psidium guajava*. *IOP Conference Series: Earth and Environmental Science* 269:012020. https://doi.org/10.1088/1755-1315/269/1/012020

Intergovernmental Panel on Climate Change (IPCC). 2006. IPCC Guidelines for National Greenhouse Gas Inventories - Volume 4 Agriculture, Forestry and other Land Use. In: Eggleston H S, Buendia L, Miwa K, Ngara T, Tanabe K, eds. Prepared by the National Greenhouse Gas Inventories Programme, IGES, Japan.

Intergovernmental Panel on Climate Change (IPCC). 2022. Summary for policymakers. In P. R. Shukla, J. Skea, R. Slade, A. A. Khourdajie, R. van Diemen, D. McCollum, M. Pathak, S.Some, P. Vyas, R. Fradera, M. Belkacemi, A. Hasija, G. Lisboa, S. Luz, & J. Malley, eds. Climate Change 2022: Mitigation of ClimateChange. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

International Coffee Organization (ICO). 2023. Coffee report and outlook 2023. Available online: https://icocoffee.org/documents/cy2023-24/Coffee_Report_and_Outlook_December_2023_ICO.pdf

Jawo TO, Kyereh D, Lojka B. 2022. The impact of climate change on coffee production of small farmers and their adaptation strategies: a review. *Climate and Development* 15(2), 93-109. https://doi.org/10.1080/17565529.2022.2057906

Jha S, Bacon CM, Philpott SM, Rice RA, Méndez VE, Läderach P. 2011. A Review of Ecosystem Services, Farmer Livelihoods, and Value Chains in Shade Coffee Agroecosystems. In: Campbell, W., Lopez Ortiz, S. (Eds) Integrating Agriculture, Conservation and Ecotourism: Examples from the Field. Issues in Agroecology – Present Status and Future Prospectus, vol 1. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1309-3_4

Jose S. 2009. Agroforestry for ecosystem services and environmental benefits: an overview. *Agrofor. Syst*. 76:1–10. https://doi.org/10.1007/s10457-009-9229-7.

Jiao L, Lu N, Fang W, Li Z, Wang J, Jin Z. 2019. Determining the independent impact of soil water on forest transpiration: a case study of a black locust plantation in the Loess Plateau, China. *Journal of Hydrology* 572:671-681. https://doi.org/10.1016/j.jhydrol.2019.03.

Jin, S., Zhang, E., Guo, H. et al. 2023. Comprehensive evaluation of carbon sequestration potential of landscape tree species and its influencing factors analysis: implications for urban green space management. *Carbon Balance Manage* 18:17. https://doi.org/10.1186/s13021-023-00238-w

Kirkham MB. 2005. WATER-USE EFFICIENCY. Encyclopedia of Soils in the Environment, 315–322. https://doi.org/10.1016/b0-12-348530-4/00441-0

Kursten E, Burschel P. 1993. CO_2 mitigation by agroforestry. *Water, Air and Soil Pollution* 70(1-4):533-544. https://doi.org/10.1007/BF01105020

Lal R, Negassa W, Lorenz K. 2015. Carbon sequestration in soil. *Current Opinion in Environmental Sustainability* 15:79-86. https://doi.org/10.1016/j.cosust.2015.09.002

Larcher W. 1994. Photosynthesis as a tool for indicating temperature stress events. In Schulze ED, Caldwell MM, eds. Ecophysiology of Photosynthesis. Springer-Verlag: Berlin, Germany, pp 261-277.

Lepeduš H, Viljevac M, Cesar V, Ljubešić N. 2005. Functioning of the photosynthetic apparatus under low and high light conditions in chlorotic spruce needles as evaluated by *in vivo* chlorophyll fluorescence. *Russian Journal of Plant Physiology* 52(2):165-170. https://doi.org/10.1007/s11183-005-0024-7

Liang S, Wang J. 2020. Aboveground biomass. In Liang S, Wang J (Eds) Advanced Remote Sensing, 2nd edn. Academic Press, London, United Kingdom, pp. 543-580.

Lin, B.B., 2007. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. *Agricultural and Forest Meteorology* 144(1-2):85–94. https://doi.org/10.1016/j.agrformet.2006.12.009

Lima PF, Filho, Pinheiro JM. 2004. Gas exchange of the umbu tree under semi-arid conditions. *Revista Brasileira De Fruticultura* 26(2):206-208. https://doi.org/10.1590/S0100-29452004000200006

Long SP. 1985. Leaf gas exchange. In Barber J, Baker NR, eds. Photosynthetic Mechanisms and the Environment. Elsevier, Amsterdam, The Netherlands, pp 453-500.

López LL, Barrientos-Priego AF, Ben-Ya'acov AD. 1999. Variabilidad genética de los bancos de germoplasma de aguacate preservados en el Estado de México. *Revista Chapingo Serie Horticultura* 5:19-23.

Lott JE. 1998. Resource capture and use in semi-arid overstorey agroforestry systems. PhD thesis, University of Nottingham.

Magnago LFS, Magrach A, Laurance WF, Martins SV, Meira-Neto JAA, Simonelli M, Edwards DP. 2015. Would protecting tropical forest fragments provide carbon and biodiversity cobenefits under REDD + ? *Global Change B*iology 21, 3455-3468. https://doi.org/10.1111/gcb.12937

Makino A, Nakano H, Mae T. 1994. Effects of growth temperature on the response of ribulose- 1,5 bisphosphate carboxilase, electron transport components and sucrose synthesis enzymes to leaf nitrogen in rice and their relationships to photosynthesis. *Plant Physiology* 105:1231-1238. https://doi.org/10.1104/pp.105.4.1231.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence-A practical guide. *Journal of Experimental Botany* 51: 659-668. https://doi.org/10.1093/jexbot/51.345.659

McCarthy HR, Pataki DE, Jenerette GD. 2011. Plant water-use efficiency as a metric of urban ecosystem services. *Ecological Applications* 21(8):3115–3127. https://doi.org/10.1890/11-0048.1

Medrano H, Bota J, Cifre J, Flexas J, Ribas-Carbó M, Gulías J. 2007. Eficiencia en el uso del agua por las plantas. *Investigaciones Geográficas* (43):63-84. https://www.redalyc.org/articulo.oa?id=17604304

Miceli-Méndez CL, Ferguson GB, Ramírez-Marcial N. 2008. Seed dispersal by cattle: natural history and applications to neotropical forest restoration and agroforestry. In Myster R. Ed. Post-Agricultural Succession in the Neotropics, Springer, New York, pp 165-191. https://doi.org/10.1007/978-0-387-33642-8_7

Moguel P, Toledo VM (1999) Biodiversity Conservation in Traditional Coffee Systems of Mexico. *Conservation Biology* 13(1):11–21. doi:10.1046/j.1523-1739.1999.97153.x

Mordor Intelligence. 2024. *Global Coffee Market. 2024 - 2029*. Available online: https://samples.mordorintelligence.com/46502/Sample%20-%20Global%20Coffee%20Market%20(2024%20-%202029)%20-%20Mordor%20Intelligence1706520790961.pdf

Mosquera-Sanchez LP, Riaño-Herrera NM; Arcila-Pulgarin J, Ponce-Davila CA. 1999. Fotosíntesis, respiración y fotorrespiración en hojas de café *Coffea* sp. *Cenicafé* 50(3):215-221

Naik SK, Sarkar PK, Das B, Singh AK, Bhatt BP. 2021. Biomass production and carbon stock in *Psidium guajava* orchards under hot and sub-humid climate. *Current Science* 120(10):1627-1635. https://doi.org/10.18520/cs/v120/i10/1627-1635

Nava D, Ramírez-Ramírez I, Peña-Valdivia CB, Díaz-Villaseñor G, González-Hernández VA. 2009. Características del intercambio de gases en hojas de guayabo. *Psidium guajava* L.). *Revista Chapingo Serie horticultura* 15(2):119-126.

Nelson DW, Sommers LE. 1980. Total Nitrogen Analysis of Soil and Plant Tissues, *Journal of Association of Official Analytical Chemists* 63(4):770–778, https://doi.org/10.1093/jaoac/63.4.770

Nestel D. 1995. Coffee in Mexico: international market, agricultural landscape and ecology. *Ecological Economics* 15(2):165-178. https://doi.org/10.1016/0921-8009(95)00041-0

Niembro RA. 1992. Árboles útiles de México: naturales e introducidos. Limusa, México, pp 206

Niinemets Ü, Kull O. 2001. Sensitivity of photosynthetic electron transport to photoinhibition in a temperate deciduous forest canopy: photosystem II center openness, non-radiative energy dissipation and excess irradiance under field conditions. *Tree Physiol* 21:899–914 . https://doi.org/10.1093/treephys/21.12-13.899

Noponen MRA., Healey JR, Soto G, Haggar JP, 2013. Sink or source-The potential of coffee agroforestry systems to sequester atmospheric CO2 into soil organic carbon. *Agric. Ecosyst. Environ.* 175, 60–68. https://doi.org/10.1016/j.agee.2013.04.012

North GB, Lynch FH, Maharaj FD, Phillips CA, Woodside WT. 2013. Leaf hydraulic conductance for a tank bromeliad: axial and radial pathways for moving and conserving water. *Frontiers in Plant Science* 4:78. https://doi.org/10.3389/fpls.2013.00078

Notaro KA, Medeiros EV, Duda GP, Silva AO, Moura PM. 2014. Agroforestry systems, nutrients in litter and microbial activity in soils cultivated with coffee at high altitude. *Sci Agric* 71:87–95. https://doi.org/10.1590/S0103- 90162014000200001.

Nutman FJ. 1937. Studies of the physiology of *Coffea arabica* L. I. Photosynthesis of coffee leaves under natural conditions. *Annals of Botany* 1:353-367.

Nygren P. 1995. Leaf CO_2 exchange of *Erythrina poeppigiana* (Leguminosae: Phaseolae) in humid tropical field conditions. *Tree Physiology* 15(2):71-83, https://doi.org/10.1093/treephys/15.2.71

Ortiz-Ceballos GC, Vargas-Mendoza M, Ortiz-Ceballos AI, Mendoza Briseño M, Ortiz-Hernández G. 2020. Aboveground Carbon Storage in Coffee Agroecosystems: The Case of the Central Region of the State of Veracruz in Mexico. *Agronomy* 10: 382. https://doi.org/10.3390/agronomy10030382

Palma-Garcia JM, Gonzales-Rebeles Islas C. 2018. Recursos arbóreos y arbustivos tropicales para una ganadería bovina sustentable. Dirección General de Publicaciones, Universidad de Colima, México.

Panhuysen S, Pierrot J. 2020. Coffee barometer 2020. Coffee Collective 2020, Belgium

Pascarella J, Aide T, Serrano M, Zimmerman JK. 2000. Land-Use History and Forest Regeneration in the Cayey Mountains, Puerto Rico. *Ecosystems* 3:217-228. https://doi.org/10.1007/s100210000021

Pham Y, Reardon-Smith K, Mushtaq S. et al. 2019 The impact of climate change and variability on coffee production: a systematic review. *Climatic Change* 156, 609-630. https://doi.org/10.1007/s10584-019-02538-y

Poorter L, Wright SJ, Paz H, Wright IJ. 2008. Are functional traits good predictors of demographic rates? Evidence from five Neotropical forests. *Ecology, 89*(7), 1908-1920. https://doi.org/10.1890/07-0207.1

Pritchard SG, Amthor JS. 2005. Crops and environmental change: an introduction to effects of global warming, increasing atmospheric CO_2 and O_3 concentrations, and soil salinization on crop physiology and yield. Food Products Press, New York.

Rahn E, Läderach P, Baca M. et al. 2014. Climate change adaptation, mitigation and livelihood benefits in coffee production: where are the synergies? *Mitigation and Adaptation Strategies for Global Change* 19:1119-1137. https://doi.org/10.1007/s11027-013-9467-x

Ramalho J, Pons TL, Groeneveld HW, Azinheira HG, Nunes MA. 2000. Photosynthetic acclimation of high light conditions in mature leaves of *Coffea arabica* L.: Role of xanthophylls, quenching mechanisms and nitrogen nutrition. *Australian Journal of Plant Physiology* 27(1):43-51. https://doi.org/10.1071/PP99013

Reigosa MS, Gonzalezy L, Souto XC, Pastoriza JE. 2000. Allelopathy in forest ecosystems. In: Narwal SS, Hoagland RE, Dilday RH, Reigosa MJ (Eds). Allelopathy in Ecological Agriculture and Forestry. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-4173-4_12

Reis R, Carneiro M, Pereira E, Furtado R, Morais L, Magalhães J, et al. 2022. Biomass components and water use efficiency in cactus pear under different irrigation systems and harvest frequencies. *Revista Brasileira De Zootecnia* 51:e20210093. https://doi.org/10.37496/rbz5120210093

Riaño NM. 1993. Fotosíntesis en hojas de café In: Centro Nacional de Investigaciones de Café, Cenicafé. Informe anual de labores de la Disciplina de Fisiología vegetal 1992-1993, pp 26-46

Rice R. 2008. Agricultural intensification within agroforestry: The case of coffee and wood products. *Agriculture, Ecosystems and Environment* 128:212–218.

Roháček K. 2002. Chlorophyll Fluorescence Parameters: The Definitions, Photosynthetic Meaning, and Mutual Relationships. *Photosynthetica* 40:13-29. https://doi.org/10.1023/A:1020125719386

Rojas-García F, De Jong B H J, Martínez-Zurimendí P, Paz-Pellat F. 2015. Database of 478 allometric equations to estimate biomass for Mexican trees and forests. *Annals of Forest Science* 72(6):835-864. https://doi.org/10.1007/s13595-015-0456-y

Romero P, Navarro JM, García F, Botía Ordaz P. 2004. Effects of regulated deficit irrigation during the pre-harvest period on gas exchange, leaf development and crop yield of mature almond trees. *Tree Physiology* 24(3):303-12. https://doi.org/10.1093/treephys/24.3.303

Romero-Alvarado Y, Soto-Pinto L, García-Barrios L, Barrera-Gaytán JF. 2002. Coffee yields and soil nutrients under the shades of *Inga* sp. vs. multiple species in Chiapas, Mexico. *Agroforestry Systems* 54:215-224. https://doi.org/10.1023/A:1016013730154

Running SW. 1976. Environmental control of leaf water conductance in conifers. *Canadian Journal of Forest Research* 6:104-112. https://doi.org/10.1139/x76-013

Rzedowski J. 1978. Vegetación de México. Limusa. México, D.F., Mexico. 432 pp.

Salazar-Figueroa R. 1985. Producción de leña y biomasa de Inga densiflora Benth en San Ramón, Costa Rica. *Silvoenergía* (CATIE) 3. Available online: https://repositorio.catie.ac.cr/handle/11554/3510

Sakshaug E, Bricaud A, Dandonneau Y, Falkowski PG, Kiefer DA, Legendre L, Morel A, Parslow J, Takahashi M. 1998. Parameters of photosynthesis: definitions, theory and interpretation of results. *Journal of Plankton Research* 20(3):603-603. https://doi.org/10.1093/plankt/19.11.1637

Sánchez-Díaz M, Aguirreolea J. 2008. Transpiración y control estomático. In Azcón-Bieto J, Talón M. (Eds) Fundamentos de Fisiología Vegetal. McGraw-Hill Interamericana, España, pp. 41-56.

Schoppach R, Sadok W. 2012. Differential sensitivities of transpiration to evaporative demand and soil water deficit among wheat elite cultivars indicate different strategies for drought tolerance. *Environmental and Experimental Botany* 84:1-10. https://doi.org/10.1016/j.envexpbot.2012.0

Schultze ED. 1986. Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. *Annual Review of Plant Physiology* 37:247-74. https://doi.org/10.1146/annurev.pp.37.060186.001335

Schussler JR, Westgate M. 1988. Relationship between water deficits, photosynthesis and kernel number in maize. *Plant Physiology Supplement* 86:75

Schwerbrock R, Leuschner C. 2017. Foliar water uptake, a widespread phenomenon in temperate woodland ferns? *Plant Ecology* 218:555-563. https://doi.org/10.1007/s11258-017-0711-4

Segura M, Kanninen M, Suárez D. 2006. Allometric models for estimating aboveground biomass of shade trees and coffee bushes grown together. *Agrofor Syst* 68:143–150. https://doi.org/10.1007/s10457-006-9005-x

Servicio Meteorológico Nacional (SMN). 2024. Gobierno de México, Retrieved from: https://smn.conagua.gob.mx/es/climatologia/informacion-climatologica/normales-climatologicas-por-estado?estado=ver

Shimshi D, Ephrat J. 1975. Stomatal Behavior of Wheat Cultivars in Relation to Their Transpiration, Photosynthesis, and Yield. *Agronomy Journal* 67:326-331. https://doi.org/10.2134/agronj1975.00021962006700030011x

Shirke PA, Pathre U. 2003. Diurnal and Seasonal Changes in Photosynthesis and Photosystem 2 Photochemical Efficiency in *Prosopis juliflora* Leaves Subjected to Natural Environmental Stress. *Photosynthetica* 41:83-89. https://doi.org/10.1023/A:1025864513663

Siles P, Centeno H, Cabezas W, Staver C, Jürgen B, Bustamante O. 2012. Preliminary Characterisation of Common Legume Tree Species as Coffee Shade for Climate Change Readiness. Proceedings of Resilience of agricultural systems against crises, Göttingen - Kassel/Witzenhausen.

Simonin KA, Limm EB, Dawson TE. 2012. Hydraulic conductance of leaves correlates with leaf lifespan: implications for lifetime carbon gain. *New Phytologist* 193(4):939-947. https://doi.org/10.1111/j.1469-8137.2011.04014.x

Sinclair TR, Devi J, Rufty T. 2017. Limited-transpiration response to high vapor pressure deficit in crop species. *Plant Science* 260:109-118. https://doi.org/10.1016/j.plantsci.2017.04

Somarriba E. 1988. Pasture growth and floristic composition under the shade of guava. *Psidium guajava* L. trees in Costa Rica. *Agroforestry Systems* 6:15-162. https://doi.org/10.1007/BF02344752

Soto-Pinto L, Romero-Alvarado Y, Caballero-Nieto J, Segura WG. 2001. Woody plant diversity and structure of shade-grown-coffee plantations in Northern Chiapas, Mexico. *Revista de Biología Tropical* 49(3):977-987.

Stegemann J, Timm HC, Küppers M. 1996. Light environment and photosynthesis of an understorey and a pioneer species from a premontane rainforest of Costa Rica. *Revista pensamiento actual* 2:61-68.

Strasser RJ, Tsimilli-Michael M, Srivastava A. 2004. Analysis of the Chlorophyll a Fluorescence Transient. In: Papageorgiou, G.C., Govindjee (Eds) Chlorophyll a Fluorescence. Advances in Photosynthesis and Respiration, vol 19. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-3218-9_12

Taiz L, Zeiger E, Moller IM, Murphy A. 2014. Plant Physiology and Development Sinauer Associates Inc., Sunderland.

Tellez O, Mattana E, Diazgranados M, Kühn N, Castillo-Lorenzo E, Lira R, Montes-Leyva L, Rodriguez I, Flores Ortiz CM, Way M, Dávila P, Ulian T. 2020. Native trees of Mexico: diversity, distribution, uses and conservation. *PeerJ* 8:e9898. https://doi.org/10.7717/peerj.9898

Terasaki Hart, D.E., Yeo, S., Almaraz, M. et al. 2023. Priority science can accelerate agroforestry as a natural climate solution. *Nature Climate Change* 13, 1179-1190. https://doi.org/10.1038/s41558-023-01810-5

Tinoco-Ojanguren C, Pearcy RW. 1995. A Comparison of Light Quality and Quantity Effects on the Growth and Steady-State and Dynamic Photosynthetic Characteristics of Three Tropical Tree Species. *Functional Ecology* 9(2): 222. https://doi.org/10.2307/2390568

Toledo VM, Moguel P. 2012. Coffee and Sustainability: The Multiple Values of Traditional Shaded Coffee. *Journal of Sustainable Agriculture* 36(3):353-377. https://doi.org/10.1080/10440046.2011.583719

Useche-Carrillo NV, Ayala-Arreola J, Campos-Rojas E, Barrientos-Priego A. 2022. Relationships between stomatal and gas exchange characteristics of the leaf blade in ‘Colín V-33’ avocado seedlings. *Revista Chapingo Serie Horticultura* 28(3):133-144. https://doi.org/10.5154/r.rchsh.2021.05.008

United States Department of Agriculture Foreign Agricultural Service (USDA FAS). 2023. Coffee annual Mexico. Mexico City. Available online: https:// https://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=Coffee%20Annual_Mexico%20City_Mexico_MX2023-0024.pdf.

Valencia V, García-Barrios L, West P, Sterling E J, Naeem S. 2014. The role of coffee agroforestry in the conservation of tree diversity and community composition of native forests in a Biosphere Reserve. *Agriculture, Ecosystems & Environment* 189:154-163. https://doi.org/10.1016/j.agee.2014.03.024

Vázquez-Torres M., Campos-Jiménez J. and Juárez-Fragoso M. 2017. *Árboles tropicales de Veracruz*. Universidad Veracruzana, Dirección Editorial.

Vázquez-Yanes C, Orozco-Segovia A. 1992. Effect of litter from a tropical rainforest on tree seed germination and establishment under controlled conditions. *Tree Physiology* 11:391-400. https://doi.org/10.1093/treephys/11.4.391

Verchot LV, Van Noordwijk M, Kandji S. et al. 2007. Climate change: linking adaptation and mitigation through agroforestry. *Mitigation and Adaptation Strategies for Global Change* 12, 901-918. https://doi.org/10.1007/s11027-007-9105-6

Vizcaíno-Bravo Q, Williams-Linera G, Asbjornsen H. 2020. Biodiversity and carbon storage are correlated along a land use intensity gradient in a tropical montane forest watershed, Mexico. *Basic and Applied Ecology* 44:24-34. https://doi.org/10.1016/j.baae.2019.12.004.

von Caemmerer S, Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376-387. https://doi.org/10.1007/BF00384257

Warrington IJ, Kanemasu ET. 1983. Corn growth response to temperature and photoperiod. 1. Seedling emergence, tassel initiation and anthesis. *Agronomy J* 75:744-754.

Weather Spark. 2024. Weather Spark: The average weather in Teocelo and Xico, Veracruz, Mexico. Databases). Retrieved from https:// https://es.weatherspark.com/y/8665/Clima-promedio-en-Teocelo-M%C3%A9xico-durante-todo-el-a%C3%B1o; https://es.weatherspark.com/y/7587/Clima-promedio-en-Xico-M%C3%A9xico-durante-todo-el-a%C3%B1o

Whiley AW. 1994. Ecophysiological studies and tree manipulation for maximisation of yield potential in avocado (*Persea americana* Mill.), PhD Thesis, University of Natal.

Wohlfahrt G, Gu L. (2015). The many meanings of gross photosynthesis and their implication for photosynthesis research from leaf to globe. *Plant, Cell & Environment* 38(12):2500–2507. https://doi.org/10.1111/pce.12569

Wolfe BT, Sperry JS, Kursar TA. 2016. Does leaf shedding protect stems from cavitation during seasonal droughts? A test of the hydraulic fuse hypothesis. *New Phytol* 212:1007-1018. https://doi.org/10.1111/nph.14087

Wong SC, Cowan IR, Farquhar GD. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282:424-426. https://doi.org/10.1038/282424a0

Xu Z, Jiang Y, Jia B, Zhou G. 2016. Elevated-CO_2 Response of Stomata and Its Dependence on Environmental Factors. *Frontiers in Plant Science* 7:657. https://doi.org/10.3389/fpls.2016.00657

Yin MH, Vargas AI, Fuentealba C, Shahid MA, Bassil E, Schaffer B. 2023. Differences in physiological and biochemical responses to short-term flooding among the three avocado (*Persea americana* Mill.) races. *Plant Physiology and Biochemistry* 196:925-939. https://doi.org/10.1016/j.plaphy.2023.02.032

Zaman-Allah M, Jenkinson DM, Vadez V. 2011. Chickpea genotypes contrasting for seed yield under terminal drought stress in the field differ for traits related to the control of water use. *Functional Plant Biology* 38(4):270-281. https://doi.org/10.1071/FP10244

Zavafer A, Mancilla C. 2021. Concepts of photochemical damage of Photosystem II and the role of excessive excitation. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* 47:100421. https://doi.org/10.1016/j.jphotochemrev.2021.100421

Zomer RJ, Bossio DA, Sommer R. et al. 2017. Global Sequestration Potential of Increased Organic Carbon in Cropland Soils. *Scientific Reports* 7:15554. https://doi.org/10.1038/s41598-017-15794-8

Table 1(on next page)

List of the seven coffee-related shade tree species selected for *in-situ* analyses.

Source: CONABIO (https://enciclovida.mx, accessed on 10/January/2023).

Species	Acronym	Selection Process/Status
Inga inicuil Schltdl. & Cham. Ex G. Don	*Ii*	Wild/Cultivated
Inga vera Willd.	*Iv*	Wild/Cultivated
Inga punctata Willd.	*Ip*	Wild/Cultivated
Erythrina americana Mill.	*Ea*	Wild/Cultivated
Psidium guajava L.	*Pg*	Wild/Cultivated
Persea schiedeana Turcz.	*Ps*	Wild
Heliocarpus appendiculatus Nees	*Ha*	Wild

Table 2(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 3(on next page)

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	

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Figure 1

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

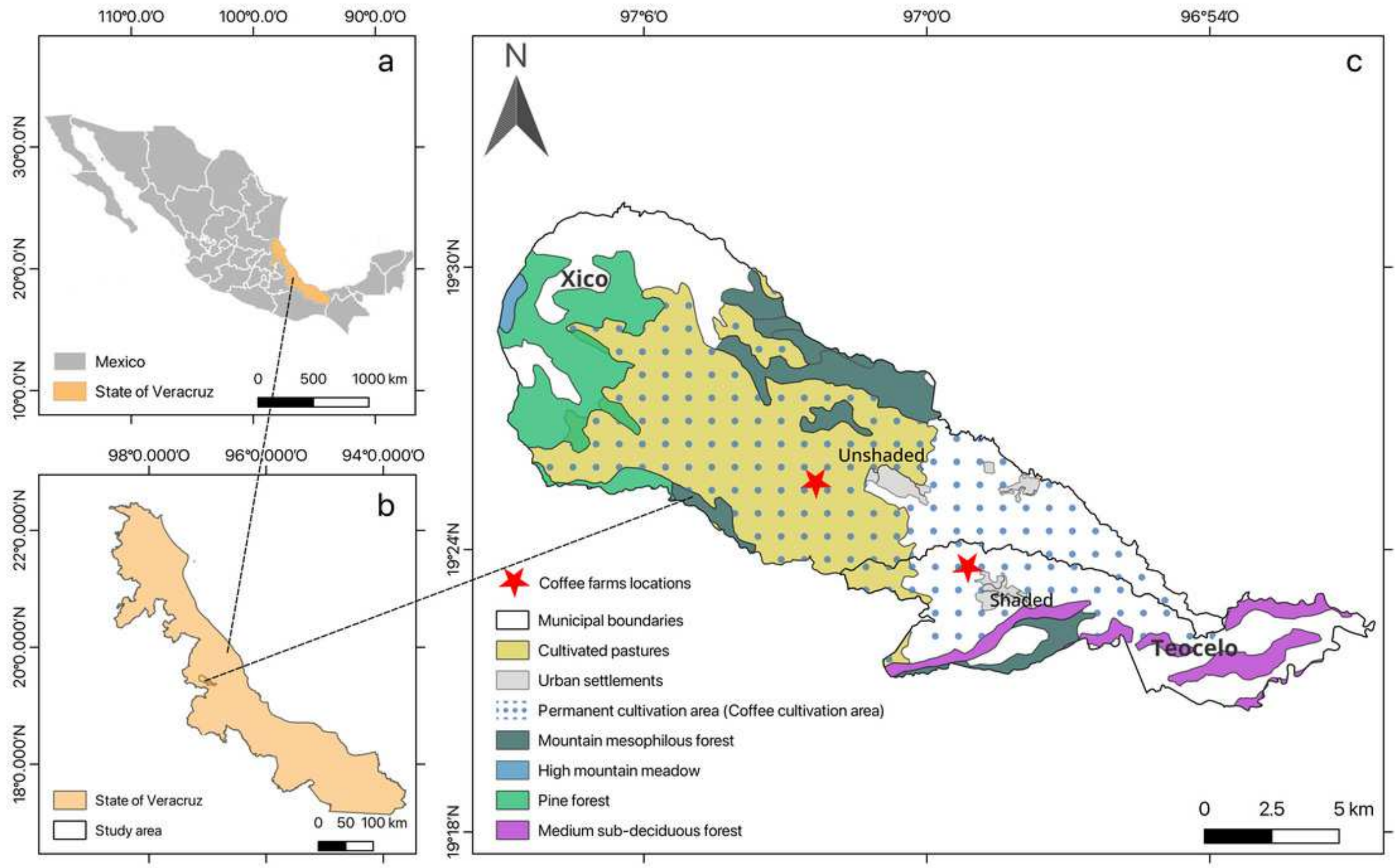

Figure 2

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.

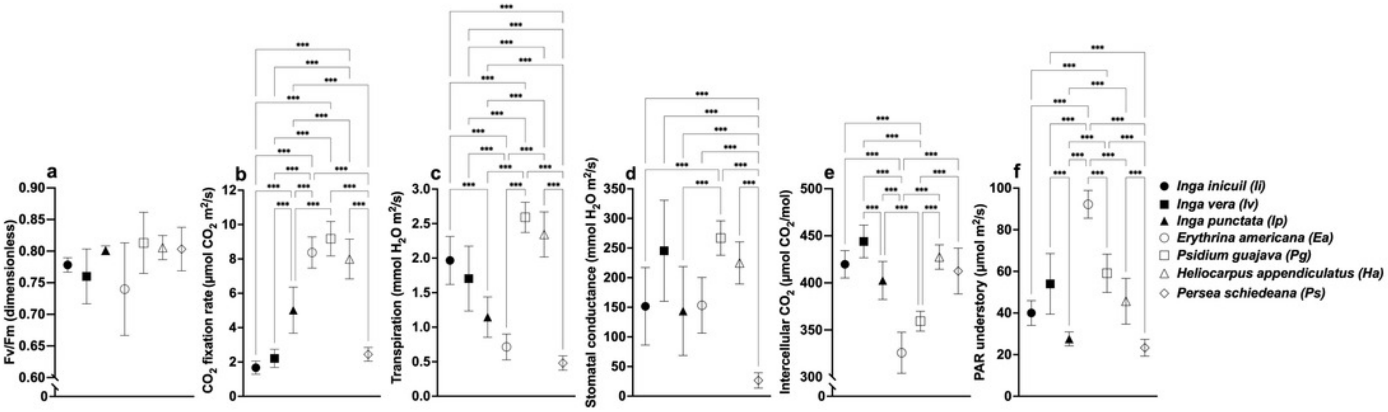

Figure 3

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.

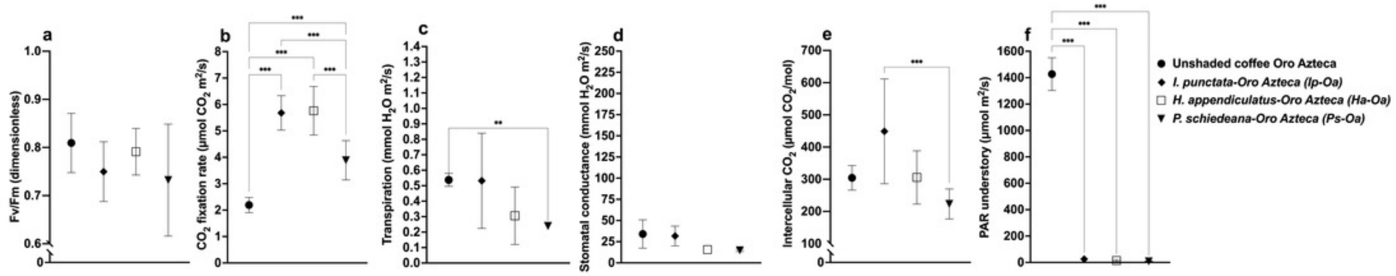

Figure 4

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.

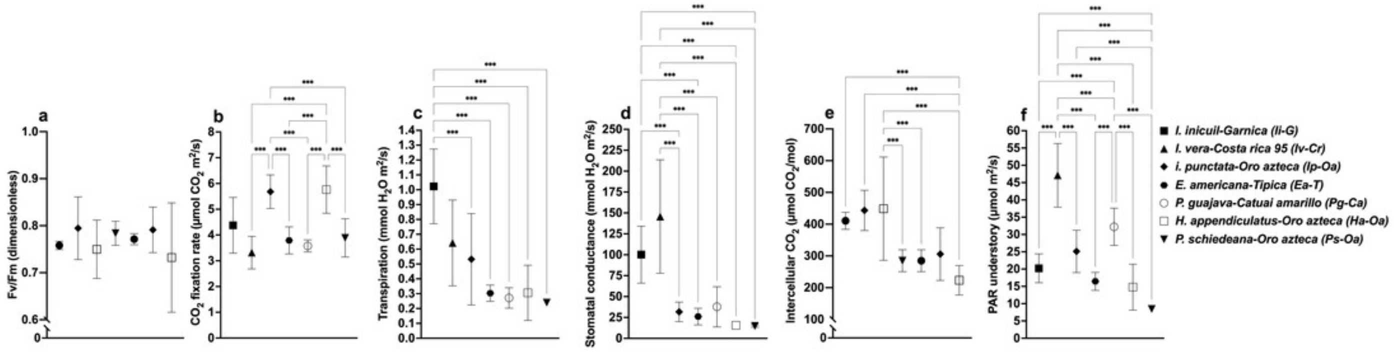

Figure 5

Instantaneous leaf water-use efficiency (ILWUE) 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.