Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Canopy stratification drives functional niche differentiation and resource use efficiency in cacao agroforestry systems

Francisco Vicente Saavedra Agramont

fsaavedraa@fcpn.edu.bo

Universidad Mayor de San Andrés

Kazuya Naoki

Universidad Mayor de San Andrés

Maritza Cornejo

Universidad Mayor de San Andrés

Carlos M. Landivar

Austrian Research Centre for Forest

Eduardo Somarriba

Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)

Stéphane Saj

Research Institute of Organic Agriculture (FiBL)

Research Article

Keywords: Carbon-water interactions, Functional traits, Microenvironmental buffering, Structural Equation Modelling, Theobroma cacao

Posted Date: July 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10351121/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Canopy stratification drives functional niche differentiation and resource use efficiency in cacao agroforestry systems

Francisco Saavedra[a,*], Kazuya Naoki[b], Maritza Cornejo[a], Carlos M. Landivar[c], Eduardo Somarriba[d], Stéphane Saj[e]

[a] Herbario Nacional de Bolivia, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, Bolivia

[b] Instituto de Ecología, Carrera de Biología, Universidad Mayor de San Andrés, La Paz, Bolivia (0000-0002-6656-6950)

[c] Department of forest growth, Silviculture and Genetics, Austrian Research Centre for Forest, Vienna, Austria (0000-0001-9895-7617)

[d] Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica (0000-0002-9371-9799)

[e] Department of International Cooperation, Research Institute of Organic Agriculture (FiBL), Switzerland (0000-0001-5856-5459)

* Corresponding author E-mail address: fsaavedraa@fcpn.edu.bo (F. Saavedra, ORCID 0000-0002-7702-7864).

Acknowledgements

The authors thank to all the staff of the Sara Ana Research and Training Centre for their support in data collection. To the staff of the Herbario National de Bolivia (LPB) and the Jardín Botánico La Paz (JBLP) for their help with leaf data processing. To Ramon Jaimez Arellano for helpful advice and comments on early version of the manuscript. This study is part of the SysCom Bolivia project (https://systemscomparison.fbl.org/project-sites/bolivia.html), supported by the Swiss Agency for Development and Cooperation (SDC), the Liechtenstein Development Service (LED), the Biovision Foundation for Ecological Development, and the Coop Sustainability Fund. The study was carried out under permission and current laws of the Government of Bolivia.

Abstract

Vertical canopy stratification generates strong microenvironmental gradients that shape plant physiological performance and resource-use strategies. Yet, the mechanistic pathways linking canopy structure, microenvironment, and trait-mediated responses remain poorly understood. In this study, we investigated how direct and indirect effects of vertical environmental heterogeneity, vegetation structure, and functional leaf traits regulates instantaneous water-use efficiency (WUE_{inst}), CO_2 assimilation rate (*A*) and transpiration rate (E) across four canopy strata in cacao agroforestry systems in Bolivia. A total of 546 leaves from 24 tree species were measured under experimental field conditions using wireless infrared gas exchange sensors. Leaf structural [Leaf area (LA), Specific leaf area (SLA)] and functional traits [Relative water content (RWC), Stomata density (SD)] were also quantified. We applied structural equation modelling to disentangle the mechanistic pathways regulating plant physiological responses across vertical strata. Our results reveal clear functional niche differentiation along the vertical canopy gradient. Upper-strata trees exhibited a resource-acquisitive strategy, characterized by increased CO_2 assimilation that outpaced transpiration resulting in a high WUE_{inst} under high photosynthetically active radiation (PAR). In contrast, lower-strata trees are immersed in buffered microenvironmental conditions, with stable humidity and temperature. Under low PAR, these trees displayed a resource-conservative strategy, with reduced *A* and E and relatively constant WUEinst. While LA and RWC increased with canopy height, SLA and SD remained stable, indicating functional convergence of leaf traits supporting carbon gain and light tolerance in the upper strata. Overall, our findings demonstrate that canopy stratification drives contrasting water-carbon use strategies through, coordinated, trait-mediated responses to environmental gradients.

Keywords: Carbon-water interactions; Functional traits; Microenvironmental buffering; Structural Equation Modelling; *Theobroma cacao*

1. Introduction

The vertical vegetation structure is a fundamental organisational feature of forest (and agroforestry) ecosystems (Lowman 2021). The presence of various vertical strata (defined by tree height) creates environmental and ecological gradients that shape community species composition, functional plant traits, and physiological activity (Baligar et al. 2021; Ge et al. 2022; Somarriba and Orozco-Aguilar 2018). Along these gradients, canopy structure generates a vertical mosaic of microenvironments, ranging from the sun-exposed upper stratum to the shaded understorey, which drives both inter- and intra-specific variability in plant growth strategies (Coble et al. 2017; Ge et al. 2022), and resource use efficiency (Lamour et al. 2023; Xing et al. 2023).

It is well established that crown position in the vertical vegetation structure determines the microenvironmental conditions that tree leaves experience. Upper strata leaves are exposed to intense photosynthetically active radiation, (PAR), higher temperatures and lower humidity, whereas understorey leaves experience less PAR, higher relative humidity, and lower and thermally stable conditions (Ge et al. 2022; Gonzáles-Armas et al. 2024). Theoretically, these contrasting microenvironmental conditions drive different selective pressures and trade-offs in the resource use strategies of plants (Ntawuhiganayo et al. 2020; Zheng et al. 2025). For instance, species in the upper stratum must deal with heat load and desiccation risk (Lowman and Schowalter 2012), potentially at the cost of efficiency, whereas understorey species must adapt their light capture strategy (Li et al. 2023).

Microenvironmental heterogeneity across vertical strata plays a central role in the expression of leaf traits related to gas exchange dynamics (Poorter and Bongers, 2006), including leaf area (LA, the entire surface area of one-side leaf), specific leaf area (SLA, the ratio between leaf area and unity weight, a measure of leaf thickness), relative water content (RWC), and stomatal density (SD, the number of stomata per unit of leaf area) (Coble et al. 2017; Lamour et al. 2023). Leaves with low SLA and high SD

(i.e., sun-adapted leaves) are better suited to withstand high PAR and high evaporative demand (Vinod et al. 2023), whereas thinner leaves with high SLA (i.e., shade-adapted leaves) tend to be more efficient under low-light conditions (Read and Stokes 2006; Yu et al. 2021). Yet, the mechanistic links between leaf traits, and physiological performance, specifically on CO_2 assimilation rate (*A*), transpiration (E), and instantaneous water-use efficiency (WUE_{inst}) remain unexplored.

Physiologically, while high transpiration (E) in the upper strata is often assumed to reduce WUE_{inst} (i.e., the short-term ratio between CO_2 assimilation and transpiration rate at leaf level) due to the high transpiration cost of preventing overheating (Baligar et al. 2021; Sun et al. 2023), plants may conversely opt for "high performance" strategies in which CO_2 assimilation rate (*A*) outpaces water loss, thus increasing WUE_{inst}. Conversely, shaded leaves in the understorey have lower E and *A* ("low physiological performance"), supporting a comparatively more stable WUE_{inst} (Cocoletzi Vásquez et al. 2022; Ge et al. 2022). Hence, the combination of environmental variation and morphological leaf traits across vertical strata may drive differences in plant physiological performance, leading to carbon and water use partitioning (Allen et al. 2024; Ray et al. 2024; Xing et al. 2023).

Cacao (*Theobroma cacao* L.) multi-strata agroforestry systems (Cocoletzi Vásquez et al. 2022; Jaimes-Suárez et al. 2022) offer a unique opportunity to test whether vertical vegetation structure drives microenvironmental conditions, leaf traits, and plant physiology. Despite extensive research on cacao shade management (Saavedra et al. 2020; Saj et al. 2025), few studies have simultaneously quantified the direct and indirect causal pathways linking such variables across the vertical strata (Sun et al. 2023).

We used Structural Equation Modelling (SEM) to quantify both the direct and indirect pathways (Cheng et al. 2022) linking microenvironmental conditions (Env), vertical vegetation structure (Vst), and leaf traits (Tst) to physiological activity across four vertical strata in a long-term agroforestry trial in Bolivia (Rüegg et al. 2025). We framed our work within the "Vertical Niche Differentiation" hypothesis (Walter

1939; Laurans et al. 2014) which proposes that vertical vegetation structure in agroforestry systems promotes carbon and water use partitioning across vertical strata (Lamour et al. 2023; Xing et al. 2023).

This study addresses a central question in plant ecophysiology: how does vertical canopy stratification regulate plant physiology responses through interacting microenvironmental conditions and functional traits? Despite the recognized importance of canopy stratification, the mechanistic pathways linking canopy structure, microenvironment, traits and physiological functions remain poorly understood in multi-strata agroecosystems. We hypothesized that: [1] microenvironmental conditions such as temperature (Tmp) and humidity (Hmd), as well as functional leaf traits, such as LA, SLA, RWC, and SD, differ between vertical strata in cacao agroforestry systems; [2] tree leaves in the high and emergent strata (with high PAR, low Hmd) would exhibit a "resource-acquisitive" strategy with high SD, low LA, SLA, and RWC, and high E, *A*, and WUE_{inst}; and [3] tree leaves in the low and middle strata with low PAR and high Hmd) would exhibit a "resource-conservative" strategy, with high LA, SLA and RWC, and low SD, E, *A* and WUE_{inst}. By integrating microenvironmental measurements, leaf functional traits, and physiological processes within a structural equation modelling framework, this study provides a mechanistic basis for understanding how vertical canopy complexity shapes plant ecophysiological responses and informs the management of cacao agroforestry systems aiming to maximize carbon gain per unit of water used (Allen et al. 2024; Ray et al. 2024).

2. Materials and methods

2.1. Study area and trial design

The study was conducted in the dry season of 2022, from April to November, at the Sara Ana Research and Training Centre in the Alto Beni region, La Paz, Bolivia (altitude 380 m, 15°27'36.60" S, 67°28'20.65" W) (https://systems-comparison.fibl.org/). The site is in the transition zone between the Andean foothills and the Amazon lowlands. The local climate is humid tropical, characterized by a dry season

from May to October and a rainy season from April to November. Long-term meteorological data (2013 - 2023) indicate an average annual rainfall of 1635±83 mm and a mean annual temperature of 27°C (Rüegg et al. 2025).

Launched in 2008, the "System Comparison trial" or SysCom trial, uses a randomized complete block design to explore ecosystem services rendered by five distinct cacao cropping systems, including: [1] conventionally (herbicide and synthetic fertilizer use) and [2] organically (manual weeding and compost use) managed cacao monocultures (MC and MO, respectively); [3] conventionally and [4] organically managed cacao agroforestry systems (AFC and AFO, respectively); and [5] dynamic cacao agroforestry systems (DAF) managed without inputs, neither organic nor conventional. Each block comprises one plot of each one of the five cropping systems, and each plot including 12 cacao genotypes (Rüegg et al. 2025). The entire trial is composed of 20 plots, each sized 48 x 48 m (2304 m^2) with a 24 x 24 m (576 m^2) sampling, central area (Annex 1).

2.2. Plot description and selection of species

This study included only the three agroforestry systems (AFC, AFO, and DAF) and their four replicates, totalling 12 plots. All plots exhibited a uniform cacao planting density of 625 trees ha^{-1} (4 m x 4 m) and a coffee density of 1250 trees ha^{-1}. The total shade tree density averaged 170 trees ha^{-1} in the AFC and AFO, and 620 tree ha^{-1} in the DAF (Rüegg et al. 2025; Saj et al. 2025).

The three agroforestry systems feature a complex vertical vegetation structure and rich diversity of tree species. The vertical vegetation is structured in four vertical strata (i.e., low, middle, high and emergent; Annex 3). Each species is assigned to a specific canopy stratum based on its practical role. For instance, timber tree species, such as *Swietenia macrophylla* and *Myroxylon balsamum*, dominate the high and emergent strata. Nitrogen-fixing tree species, such as *Inga spp*. and *Erythrina spp*., prominent in AFC and AFO, and dominate the high or emergent strata. Fruit tree species, such as *Citrus spp., Garcinia humilis*,

159 *Nephelium lappaceum*, and *Theobroma grandiflorum*, populate the middle stratum. *Theobroma cacao*
160 and *Coffea arabica cv. typica*, occupy the lower stratum (with coffee being interplanted with cacao and
161 its maximum height maintained just below the base of the cacao crown). Cacao and coffee pruning
162 follow a species-specific pruning schedule, while shade tree species are pruned according to the cacao
163 annual physiological cycle (Saj et al. 2025). For this study, we selected the five best-yielding cacao
164 genotypes from the trial (III 13, III 06, IIa 22, TSH 565, and ICS 1), one shrub (*Coffea arabica*), and 18 of
165 the most cultivated tree species in the study region (Annex 2).

166 *2.3. Data collection*

167 Data collection was conducted in April, August and November 2022, two months after the last pruning
168 of the high and emergent canopy strata and one month after the pruning of cacao trees. Adult trees in
169 each AFC, AFO and DAF system were selected for measurements. From each tree, we selected one
170 mature and healthy leaf from the top of each tree's crown. While taking care not to detach the leaf from
171 the plant, we placed the selected leaf inside a transparent 3 L plastic container fitted with infrared
172 wireless sensors synchronized via Bluetooth to a portable datalogger (SPARK LX,
173 https://www.pasco.com/products; Saavedra et al., 2020). This setup enabled real-time measurement of
174 PAR, Tmp, Hmd, *A* and E over a five-minute period. Following measurement, leaves were detached from
175 the branch and transported to the laboratory for trait analysis. To reduce variability caused by cloud
176 cover, we avoided data collection on rainy days or when leaves were wet (Saavedra et al. 2020). Data
177 from the five-minute intervals were averaged to produce leaf-level values. In total, measurements were
178 recorded from 546 leaves from 24 tree species, in four replicates of three agroforestry systems (AFC,
179 AFO, and DAF).

180 *2.3.1. Microenvironmental conditions*

A PASCO Wireless Weather Sensor (PS-3209, https://www.pasco.com/products) was used to measure PAR, 0 to 2400 µmol/m²/s, Tmp (±0.2 °C), and Hmd (±2%) outside the plastic bag, immediately before placing the sensor inside the plastic bag (Saavedra et al. 2020).

2.3.2. Physiological activity measurements

For physiological measurements, the same infrared wireless weather sensor (PS-3209, https://www.pasco.com/products) was used to record absolute air humidity, which is an indicator of E. Additionally, a wireless CO_2 sensor (PS-3208, https://www.pasco.com/products), calibrated to 400 ppm, monitored *A*. To calculate hourly transpiration and CO_2 assimilation rate per leaf area, the raw data were divided by leaf area (cm²) and multiplied by 3600 seconds (Pipitsunthonsan et al. 2018; Saavedra et al. 2020). At the leaf level, WUE_{inst} was estimated as the ratio of CO_2 assimilation to water vapor loss (Farquhar and Richards 1984; Medrano et al. 2015).

2.3.3. Leaf traits measurements

Four key functional leaf traits were measured: [1] Leaf Area (LA, cm²), defined as the one-sided projected surface area of the leaf (Pérez-Harguindeguy et al. 2013); [2] Specific Leaf Area (SLA, $cm^2\ g^{-1}$) describes the leaf area per unit of dry leaf biomass (Torrez et al. 2013); [3] Relative Water Content (RWC, $g\ g^{-1}$), which measures the relative amount of water in a leaf compared to its biomass and is often used as an indicator of plant water status (Afzal et al. 2017); and [4] Stomatal Density (SD, number of stomata per leaf area in mm^{-2}), and is involved in water flow and gas exchange (Lebrija-Trejos et al. 2010).

We measured LA and SLA following the protocol described by Pérez-Harguindeguy et al. (2013), which involved drying leaves for two days in an electric dryer at the Herbario Nacional de Bolivia. Dried leaves were then scanned, and leaf area was estimated using the ImageJ software. Stomatal density was assessed by creating transparent nail polish impressions of the leaf cuticle (Brewer 1992).

Microphotographs of these impressions were taken with a 40x trifocal optical microscope equipped with a UCMOS 03100 KPA camera. Stomata were counted using OMAX TopView 3.7 software (Daymond et al. 2011). For RWC, fresh leaf mass was measured in the field and dry mass in the laboratory. Relative water content was calculated following the protocol described by Afzal et al. (2017).

2.4. Statistical analysis

2.4.1. Database preparation

The dataset comprised 11 variables measured in 546 trees (Table 1, Figure 1), including five cacao genotypes, coffee, and 18 tree species (Annex 2) in 12 plots (three agroforestry systems, each replicated four times). As predictor variables, we used the following: [1] microenvironmental conditions (Tmp and Hmd), [2] functional leaf traits (LA, SLA, RWC, and SD), and [3] vertical vegetation structure [PAR, and one new, derived variable: (a) vertical strata (Str), representing the number of vertical strata categorised into four height classes to describe canopy complexity (Annex 3)]. As response variables, we used: E (Lin et al., 2010), *A* (Baligar et al. 2021), and WUE_{inst} (Medrano et al. 2015).

Outliers (a few) were identified and removed. Non-collinearity between the predictor variables was assessed using Pearson's correlation tests. After these quality checks, the variables were transformed as needed using a Box-Cox power transformation to improve normality and reduce heteroscedasticity (Box and Cox 1964). Variable transformations were estimated using maximum likelihood for each predictor. Subsequently, the variables were standardised to a zero mean and unit variance to ensure scale comparability and stable model estimation. These steps improve parameter interpretability and convergence in multivariate SEM frameworks (Schielzeth 2010).

2.4.2. Defining vertical vegetation structure

Vertical strata were defined using a data-driven approach based on change-point detection. Specifically, the ordered distribution of maximum tree size was analysed using mean change-point detection

implemented in the change point package in R, applying binary segmentation with three predefined change points (Killick and Eckley 2014). The resulting thresholds were used to classify individuals into four vertical strata differing in botanical composition and species-specific population density (i.e., low, middle, high, and emergent):

1. The low stratum (ranging from 1.2–4.6 m) was dominated by *Coffea arabica*, five genotypes of *Theobroma cacao* (III 13, III 06, IIa 22, THS 565, ICS 1), *Theobroma grandiflorum* and *Nephelium lappaceum*.
2. The middle stratum (4.6–7.5 m) was represented by four cacao genotypes (III 13, III 06, IIa 22, ICS 1), *Garcinia gardneriana*, *Averrhoa carambola*, *Cedrela fissilis*, *Salacia macrantha*, *Theobroma grandiflorum*, *Mangifera indica*, *Nephelium lappaceum*, *Amburana cearensis*, and *Guarea guidonia*.
3. The high stratum (7.6–11.5 m) was composed of *Erythrina fusca*, *Swietenia macrophylla*, and two species of *Inga* spp.
4. The emergent stratum (11.5–16 m) contained *Erythrina fusca*, *Centrolobium microchaete*, two species of *Inga* spp., *Stryphnodendron purpureum*, *Swietenia macrophyla* and *Myroxylon balsamum*. The number of trees and species richness within each stratum are summarised in Annex 3.

2.4.3. Structural Equation Modeling (SEM)

A conceptual structural equation model (SEM) was developed to quantify the relative contributions of direct and indirect relationships (Fan et al. 2016; Ray et al. 2024) linking latent variables, such as microenvironmental variables (Env), vegetation structure (Vst), and functional leaf traits (Trt), to tree physiological processes (Figure 1). The model assumed that Env, defined by observed variables (Tmp and Hmd), exerts direct effects on Vst, characterized by (PAR and Str) and on Trt, including (LA, SLA, RWC, and SD). These factors indirectly influence plant leaf physiological activity (WUE_{inst}, *A*, and E). We

proposed that Vst and Trt have direct (one-way) effects on WUE_{inst}, *A*, and E, while assuming reciprocal (two-way) interactions between Vst and Trt.

SEMs were fitted using robust maximum likelihood estimation (MLR) to account for potential deviations from multivariate normality (Maydeu-Olivares 2017). The model specification tested the vegetation structure and leaf trait as latent variables on physiological responses (WUE_{inst}, A, and E), with the microenvironment designated as an exogenous latent predictor. Measurement models were fitted simultaneously to define the relationships between latent constructs and their observed indicators.

Model performance was evaluated using standard fit statistics, including the Comparative Fit Index (CFI) and Root Mean Square Error of Approximation (RMSEA) (Fan et al. 2016). The results included standardised path coefficients and factor loadings, for each latent variable and observed indicator. SEM analyses were conducted using the R package "lavaan" (Rosseel 2012) (v.0.6-19).

2.4.4. Linear mixed models

We used Linear Mixed Models (LMMs) to analyse the effects of predictor variables (vegetation structure, leaf traits, and microenvironment) on response variables (WUE_{inst}, A, and E), aiming at identifying patterns across vertical strata. For each response variable and vertical stratum, we constructed a full LMM including all predictor variables identified in the SEM as fixed effects, with species included as a random effect. Reduced or best models were derived by model simplification using the dredge function from the "MuMIn" package in R (Bartoń 2022). Model selection was based on comparisons of the corrected Akaike Information Criterion (AIC) (Symonds and Moussalli 2011), considering the simplest model among all models within 2 AIC units of the best-fitting model as equally plausible (Annex 4) (Burnham et al. 2011).

Additionally, to compare study variables between vertical strata, we used β-coefficients with 95% CI from Linear Models (LM), setting the significance threshold at 0.05. All analyses were performed using R statistical software version 4.5.2 (R Core Team, 2019).

3. Results

3.1. Factors influencing water use efficiency, CO_2 assimilation, and transpiration

3.1.1. Model fit and evaluation metrics

The fitted SEM models showed comparable global fit indices. These model indices indicate moderate but not optimal fit, suggesting that while the specified SEM frameworks capture important relationships, notable residuals remain unexplained. For WUE_{inst} RMSEA values were 0.168 (90% CI [0.157–0.179]) and CFI = 0.717. For A, RMSEA values were 0.171 (90% CI [0.160–0.182]) and CFI = 0.718. Finally, for E, RMSEA was 0.180 (90% CI [0.180–0.192]) and CFI = 0.704. In all models RMSEA exceed the commonly recommended cut-off of 0.06 for good fit and CFI values between 0.70 and 0.71 fall short of the widely accepted thresholds of 0.90 or higher for adequate fit. Such results suggest that additional pathways or nonlinear effects should be considered to improve model performance. Nonetheless, the internal consistency of the measurement components and the consistency of patterns across physiological processes support the substantive insights drawn from the models.

3.1.2. Comparative analysis

Across all fitted SEM models (Table 1, Figure 2), microenvironmental variables (temperature, [Tmp], and humidity, [Hmd]), vegetation structure (Photosynthetically Active Radiation [PAR], and vertical strata [Str]), and leaf traits (Leaf Area [LA]; Specific Leaf Area [SLA]; Relative Water Content [RWC]; and Stomatal Density [SD]) consistently emerged as significant predictors of plant physiological performance. Vertical strata (Str) showed uniformly strong positive effects on instantaneous water-use efficiency (WUE_{inst}), CO_2 assimilation rate (*A*), and transpiration rate (E), highlighting their role as a major determinant of physiological activity and leaf traits. However, differences between models revealed

functional trade-offs. For instance, WUE_{inst} benefited from vertical vegetation structure under favourable microenvironmental conditions, CO_2 assimilation responded primarily to both structural and leaf trait characteristics, while the transpiration rate was more sensitive to trait constraints (Figure 2).

3.1.3. Structural Equation Model for WUE_{inst}

SEM for WUE_{inst} described a network of positive direct and indirect effects linking leaf trait factors and vegetation structure to physiological responses. The strongest associations included significant positive effects of vertical strata gradients with standardised coefficients above 0.8 while microclimate (Tmp) reach it 0.70 on WUE_{inst}. RWC had a strong positive effect, whereas SD and SLA showed weaker positive and negative influences, respectively (Table 1). Microenvironmental variables exhibited variable and occasionally negative effects (Hmd), indicating potential trade-offs between CO_2 assimilation and water conservation. A positive effect (0.51) was found between vegetation structure and leaf traits (Figure 2a).

3.1.4. Structural Equation Model for CO_2 assimilation

The CO_2 assimilation model underscored vegetation structural complexity and leaf traits as dominant drivers, with standardised path coefficients nearing 0.85 on key paths. LA and RWC exerted strong positive dominant influences, and SLA and SD maintained low negative and positive relationships with CO_2 assimilation, respectively (Table 1). Microenvironmental variables (Hmd and Tmp) showed contrasting facilitative and limiting effects, reflecting their dual role in carbon uptake. This model emphasised the effect of the vertical vegetation structure on CO_2 assimilation. The correlation between vegetation structure and leaf traits was 0.76 (Figure 2b).

3.1.5. Structural Equation Model for Transpiration rate

The transpiration model highlighted the constraining role of leaf traits in controlling water loss. Leaf trait attributes and vertical strata displayed strong positive path coefficients (≥ 0.70) influencing transpiration. RWC had a strong positive effect, whereas SD and LA showed a weaker positive effect,

and SLA had negative effects (Table 1). Hmd presented more variable negative paths, suggesting water conservation and carbon assimilation trade-offs under varying microclimatic conditions. Vegetation structure and leaf traits were correlated at 0.49 in this model (Figure 2c).

3.2. Effects of microenvironment, vegetation structure, and leaf traits on physiological activity across vertical strata

We observed turnover in the predictor variables influencing the three physiological processes studied. Across all models and strata, PAR and Hmd consistently had significant effects. In the low and middle strata, microenvironmental factors (PAR and Hmd) affected WUE_{inst}, whereas PAR and leaf traits (LA, SLA and RWC) were the dominant drivers in high and emergent strata (Table 2, Figure 3). For CO_2 assimilation, leaf traits (LA, SLA and SD) were the primary drivers in the low and middle strata, whereas LA dominated across all strata (Table 3, Figure 4). For transpiration, the microenvironment (Hmd) and leaf traits (LA) drove water loss at low to high strata, with LA as the main driver in the emergent stratum (Table 4, Figure 5).

3.3. Changes in microenvironment, vegetation structure, leaf traits and physiological activity by vertical strata

With contrasting patterns, physiological variables WUE_{inst} and E, reacted for two different strata groups: low and middle against high and emergent strata. No significant differences were observed between strata for CO_2 assimilation. PAR exhibited a positive increase with tree height. Humidity and temperature remained consistent across strata. LA and RWC displayed positive increase with vertical strata, whereas SLA and SD remained stable (Figure 6).

4. Discussion

Our SEMs revealed a strong positive correlation between vegetation structure and leaf traits across all physiological components, indicating that the structural heterogeneity of the vertical strata promotes

342 niche and resource use partitioning within the canopy, as was found in previous studies (Xing et al.
343 2023). However, the moderate model fit indices (RMSEA = ~0.17) reflect potential unmodelled
344 complexity (Hu and Bentler 1999; Kline 2023). This suggests that additional latent variables such as leaf
345 age and longevity (Miyaji et al. 1997), branch hydraulic architecture (Meinzer et al. 2008) or biochemical
346 composition are likely to contribute to the physiological responses observed (Kline 2023; Sun et al.
347 2023). Thus, while our conceptual framework captures major causal drivers, physiological dynamics are
348 governed by a broader set of interacting environmental and functional mechanisms that have not yet
349 been studied (which may be influenced by the regular tree pruning undertaken in the trial; Saj et al.
350 2025).

351 We prioritised a theoretical model structure based on established ecophysiological frameworks over
352 post-hoc modifications solely to improve statistical fit. Furthermore, gas exchange measurements were
353 conducted using portable field sensors (PASCO). While these allowed for extensive sampling coverage
354 (546 trees) critical for community-level analysis, they may have lower sensitivity at low photosynthetic
355 rates compared to high-precision benchtop infra-red gas analyser. However, our focus was on relative
356 differences and functional trends across strata rather than absolute physiological parameterisation. The
357 consistency of our PAR-response curves with literature values (e.g., Baligar et al. 2021) supports the
358 robustness of the comparative patterns observed.

359 *4.1. Vertical vegetation structure as a driver of physiological niche differentiation*

360 Our study shows that the vertical vegetation structure of the agroforestry canopy acts as a fundamental
361 driver of physiological functioning, creating distinct ecological niches ruled by the interplay between
362 microclimatic conditions and plant functional traits (Laurans et al., 2014). The strongest driver of
363 physiological variation was photosynthetic active radiation (PAR), which increased with tree height and
364 drove different responses in *A*, E, and WUE_{inst} across vertical strata, consistent with patterns reported by

Vinod et al. (2023). Although PAR's influence on *A* and E is well known in tropical trees (Baligar et al. 2021; Lamour et al. 2023), our results show that PAR alone is insufficient to enhance carbon gain at the highest canopy positions, where evaporative demand increases water loss and WUE_{inst} (De Pauw et al. 2022). Leaf traits such as LA and RWC, which were clearly differentiated across strata, reflect strong vertical plasticity, whereas SLA and SD remained constant across vertical strata. This finding aligns with vertical niche differentiation theory (Poorter 2007) indicating that understorey plant species (such as cacao) reduce carbon gain to preserve water status under low light availability by developing a "resource-conservative" strategy (Cocoletzi Vásquez et al. 2022) and reinforcing their classification as shade-tolerant species (Baligar et al. 2021). In contrast, dominant canopy trees function as "resource-acquisitive", supporting their classification as sun-light demanding species (Sun et al. 2023; Yu et al. 2021).

4.2. Physiological response to microenvironmental conditions

Contrary to the theory that high radiation loads in tropical canopies induce physiological stress and reduce water use efficiency (Mulkey et al. 1996), our results revealed that trees in the high and emergent strata exhibited significantly higher WUE_{inst} than those in the lower strata. This finding challenges the paradigm that WUE decreases under high evaporative demand (VPD, that measure the drying power of the air) and low humidity (Gonzáles-Armas et al. 2024; Hatfield and Dold 2019). Instead, it highlights a mechanism of vertical niche differentiation (Poorter 2007), in which dominant canopy species such as *Centrolobium microchaete* and *Erythrina spp*., act as resource-acquisitive species. Although these tree species exhibited high E driven by exponential increases in PAR, their photosynthetic machinery was sufficiently robust to maximize *A* at a rate that outpaced water loss, a finding consistent with recent studies (Gonzáles-Armas et al. 2024; Wu and Wang 2024). This capacity to maintain high *A* under high PAR suggests that emergent species possess high carboxylation capacities

(Rozendaal et al. 2006) and are adapted to exploit the abundant solar energy of the upper canopy, rather than merely tolerating it (Baligar et al. 2008).

In contrast, the low and middle strata appear to function as a "sheltered niche" defined by a decoupling of the PAR and thermal environments. A critical finding of this study was the relative stability of Tmp and Hmd across the vertical strata, despite the steep gradient in PAR. This indicates that the multi-strata canopy acts as an effective microclimatic buffer (Niether et al. 2020), protecting the understorey species from the hydraulic stress associated with high temperatures and VPD (Gonzáles-Armas et al. 2024). In this buffered environment, understorey species adopt a "resource-conservative" strategy. Limited by light availability, these species prioritise persistence and hydraulic safety over maximum carbon gain, maintaining lower but stable physiological rates (Almeida and Valle 2007; Lahive et al. 2019). This physiological divergence aligns with the "leaf economics spectrum" (Reich and Flores-Moreno 2017; Wright et al. 2004), placing upper-canopy trees at the quick-return end of the spectrum and understorey species at the slow-return end. Furthermore, these patterns support the "environmental filtering" hypothesis regarding community assembly (Sommer et al. 2014). The upper canopy acts as a severe abiotic filter, subjecting plants to high radiation and atmospheric demand. The observation that emergent species, despite their taxonomic diversity, converged on a similar strategy of high WUE_{inst} and maintained stable morphological traits (SLA and SD) suggests functional convergence. These species appear to have evolved a shared set of sun-adapted strategies necessary to maintain hydraulic integrity in an unbuffered environment (Poorter et al. 2007). Conversely, the environmental buffering observed in the lower strata reduces these abiotic selective pressures. This functional moderation allows the understorey community to diverge from the strict hydraulic requirements of the canopy top, facilitating the coexistence of species with traits for shade tolerance and nutrient conservation rather than stress resistance (Daymond et al. 2011).

4.3. Leaf trait effects on plant physiology

A key insight from our study is that trait adjustment did not fully match the microenvironmental niches created along the vertical vegetation structure. SLA and SD remained relatively constant across vertical strata, in contrast to other shade-tolerant species, where these traits display strong vertical plasticity (Yu et al. 2021; Coble et al. 2017). In contrast, LA and RWC were clearly differentiated along vertical strata, reflecting leaf-level hydraulic adjustments that support turgor maintenance under high VPD conditions (Ntawuhiganayo et al. 2020, Read and Stokes 2006). Thus, in our case, plants appear to rely more on hydraulic buffering than on modifying tissue density or gaseous diffusion capacity to cope with vertical microclimatic stress.

The contrasting responses observed in WUE_{inst} and E between the low and middle versus the high and emergent vertical strata reveals a strong hydraulic trade-off, that improves carbon acquisition at the expense of water conservation at high and emergent strata. A similar trade-off has been reported in vertically structured forests where higher light intensities facilitate carbon uptake but impose water-loss under high evaporative demand (Ge et al. 2022; Hatfield and Dold 2019). Our SEM results further demonstrate that microenvironmental stress gradients are the primary cause of this divergence, as humidity exerts significant positive effect on E and WUE_{inst} in the high and emergent strata. These results support mechanistic frameworks describing how the microclimate shapes leaf-level energy balance and water use in multi-strata vegetation systems (Lamour et al. 2023).

Surprisingly, CO_2 assimilation did not vary significantly across vertical strata, despite sharp changes in PAR and humidity. This decoupling suggests that carbon assimilation reaches a physiological threshold under shade conditions beyond which additional radiation provides no substantial photosynthetic benefit (Amissah et al. 2015). This could be explained by biochemical limitations such as Rubisco activation and chloroplast density, typical in shaded leaves (Baligar et al. 2021). A similar pattern of CO_2 assimilation rate across vertical strata has been observed in other understorey species under chronic

light limitation (Ray et al. 2024). This finding underscores the understorey conservative carbon-use strategy and may explain the limited trait plasticity in SLA and SD.

Interestingly, physiological rates (*A*, E, and WUE_{inst}) and some leaf traits, such as LA and RWC, showed clear changes along the vertical vegetation structure. In contrast, both SLA and SD remained relatively stable across the vertical strata. These results contrast with classical ecological theory, which predicts that sun leaves (upper canopy) should be thicker (lower SLA) and have higher stomatal density than shaded leaves (Wright et al. 2004). The stability of SLA in this system may be driven by species turnover rather than by intraspecific plasticity. The change in species composition from broad-leaved species in the understorey to legume-like leaves in the overstorey might result in a convergence of functional traits. Alternatively, it suggests that the buffered microclimate in these multi-strata systems reduces the selective pressure for distinct anatomical modifications typically seen in harsh, open environments (Rozendaal et al. 2006).

4.4. Implications for agroforestry design and management

Overall, this study provides the first empirical evidence for distinct, physiologically-defined, species groups across strata in cacao agroforestry systems, showing that high and emergent trees operate under water-loss conditions with increased efficiency, whereas low and middle strata maintain a more favourable balance between carbon gain and water loss. These findings offer a novel mechanistic understanding of physiological activity in complex vertical environments and contribute to a growing body of evidence showing that microclimatic heterogeneity, structural characteristics, and leaf traits fundamentally shape ecophysiological performance in tropical agroforestry systems (Allen et al. 2024; Cocoletzi Vásquez et al. 2022).

The confirmation that upper-canopy trees function as highly efficient carbon sinks rather than mere competitors with the crops in the understorey, supports the need for a productive emergent stratum.

Our findings indicate that maintaining high-efficiency, timber or N-fixing species in this stratum is critical for preserving hydraulic protection and system-level carbon gain.

Simultaneously, the management (regular and intensive pruning and lopping) of the middle stratum to facilitate light penetration allows for adequate photosynthesis, flowering and yield production of the crop species in the low stratum (cacao and coffee) without disrupting the microclimatic buffering that protects the understorey from drought (Saj et al. 2025). This validates the multifunctional species management as an effective strategy for climate-resilient agroforestry, balancing the trade-off between sustainability and stress resilience in the face of changing climatic conditions (Keprate et al. 2024). Additionally, these physiological insights provide a mechanistic interpretation of the need and effects of canopy management strategies, such as pruning and lopping, tested in the SysCom trial (Saj et al. 2025; Rüegg et al. 2025). Pruning may influence carbon and water use strategies, with direct effects on crop productivity and resource use efficiency. More research is needed to assess such potential effects (Forrester et al. 2012; Maurin and DesRochers 2013).

5. Conclusion

Our study argues that vertical vegetation structure in cacao agroforestry systems is driven by distinct carbon and water use strategies, which are primarily modulated by PAR-humidity gradients and hydraulic traits (LA, RWC). A distribution resource-acquisition strategy was identified, where shaded lower-strata trees maintained conservative water use, whereas sun-exposed upper-strata trees prioritised carbon acquisition despite higher water loss. Despite the capacity of LA and RWC to adapt to the microclimate, the limited plasticity of SD and SLA suggests that the canopy functions as a buffer against extremes, thereby promoting trait differentiation. The findings of the structural equation modelling indicated that the vegetation structure may exert a significant influence on physiological pathways. However, the moderate model fit suggests the need for future research to identify and

incorporate additional factors (such as leaf age and hydraulic architecture, both potentially influenced by pruning) that would improve the fit of these models. Overall, our results clarify how canopy spatial heterogeneity can regulate tree physiology in multi-strata agroforestry systems, highlighting vertical niche differentiation as a potential fundamental driver of the functioning of these agro-ecosystem.

Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT Authorship contribution statement

Francisco Saavedra (FS): Conceptualization; FS and Kazuya Naoki (KN): Study design and methodology; FS, KN and Maritza Cornejo (MC): Data collection; FS, KN and Carlos M. Landivar (CML): Data curation, formal analysis and interpretation of results; FS: Writing - original draft; FS, KN, MC, CML, Eduardo Somarriba (ES) and Stéphane Saj (SS): Commented, review and edited the original draft of the manuscript; FS, SS: Conceptual model visualization; SS: Project administration; SS: Funding acquisition.

Funding sources

This study is part of the SysCom Bolivia project (https://systemscomparison.fbl.org/project-sites/bolivia.html), supported by the Swiss Agency for Development and Cooperation (SDC), the Liechtenstein Development Service (LED), the Biovision Foundation for Ecological Development, and the Coop Sustainability Fund.

Declaration of generative AI use

During the preparation of this manuscript the author(s) used Gemini in order to review and summarize complex literature and solving coding bugs; chatGPT to provide support for content organization and improving language and readability. After using this AI tools, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

Appendix A. Supporting information

Data availability

Data will be made available on request

References

Afzal A, Duiker SW, Watson JE (2017) Leaf thickness to predict plant water status. Biosyst Eng 156: 148-156. https://doi.org/10.1016/j.biosystemseng.2017.01.011

Almeida AAFD, Valle RR (2007) Ecophysiology of the cacao tree. Braz J Plant Physiol 19(4): 425-448. https://doi.org/10.1590/S1677-04202007000400011

Allen SL, Robayo LA, Martin CD, López Ganem J (2024) Productivity, soil health, and tree diversity in dynamic cacao agroforestry systems in Ecuador. Land 13(7): 959. https://doi.org/10.3390/land13070959

Amissah L, Mohren GM, Kyereh B, Poorter L (2015) The effects of drought and shade on the performance, morphology and physiology of Ghanaian tree species. PLoS One 10(4): e0121004. https://doi.org/10.1371/journal.pone.0121004

Baligar VC, Bunce JA, Machado RCR, Elson MK (2008) Photosynthetic photon flux density, carbon dioxide concentration, and vapor pressure deficit effects on photosynthesis in cacao seedlings. Photosynthetica 46(2): 216-221. https://doi.org/10.1007/s11099-008-0035-7

Baligar VC, Elson MK, Almeida AAF, de Araujo QR, Ahnert D, He Z (2021) The impact of carbon dioxide concentrations and low to adequate photosynthetic photon flux density on growth, physiological traits, and nutrient use efficiency of cocoa genotypes. Agronomy 11(2): 397. https://doi.org/10.3390/agriculture11020397.

524 Bartoń K. (2022) MuMIn: multi-model inference. R package version 1.46. 0.

525 Box GE, Cox DR (1964) An analysis of transformations. J R Stat Soc Ser B Stat Methodol 26(2): 211-243.

526 Brewer CA (1992) Responses by stomata on leaves to microenvironmental conditions. Tested studies for
527 laboratory teaching, 67-75. In: Goldman CA (ed) Tested studies for laboratory teaching, Volume 13
528 Proceedings of the 13th Workshop/Conference of the Association for Biology Laboratory Education
529 (ABLE). pp 191.

530 Burnham KP, Anderson DR, Huyvaert KP (2011) AIC model selection and multimodel inference in
531 behavioral ecology: some background, observations, and comparisons. Behav Ecol Sociobiol 65(1): 23-
532 35. https://doi.org/10.1007/s00265-010-1029-6

533 Cheng Y, Leung LR, Huang M, Koven C, Detto M, Knox R, Bisht G, Bretfeld M, Fisher RA (2022) Modeling
534 the joint effects of vegetation characteristics and soil properties on ecosystem dynamics in a Panama
535 tropical forest. J Adv Model Earth Syst 14(1): e2021MS002603. https://doi.org/10.1029/2021MS002603

536 Coble AP, Fogel ML, Parker GG (2017) Canopy gradients in leaf functional traits for species that differ in
537 shade tolerance. Tree Physiol 37(10): 1415–1423. https://doi.org/10.1093/treephys/tpx060

538 Cocoletzi Vásquez E, Hipólito-Romero E, Ricaño-Rodríguez J, Ramos-Prado JM (2022) Ecophysiological
539 plasticity of *Theobroma cacao* L. clones in response to the structure and microclimate of agroforestry
540 systems in Mexico. Bot Sci 100(4): 960-976. https://doi.org/10.17129/botsci.2925

541 Daymond AJ, Tricker PJ, Hadley P (2011) Genotypic variation in photosynthesis in cacao is correlated
542 with stomatal conductance and leaf nitrogen. Biol Plant 55: 99–104. https://doi.org/10.1007/s10535-
543 011-0013-y

De Pauw K, Sanczuk P, Meeussen C, Depauw L, De Lombaerde E, Govaert S, Vanneste T, Brunet J, Cousins SAO, Gasperini C, Hedwall PO, Iacopetti G, Lenoir J, Plue J, Selvi F, Spicher F, Uria-Diez J, Verheyen K, Vangansbeke P, De Frenne P (2022) Forest understorey communities respond strongly to light in interaction with forest structure, but not to microclimate warming. New Phytol 233(1): 219-235. https://doi.org/10.1111/nph.17803

Fan Y, Chen J, Shirkey G, John R, Wu SR, Park H, Shao C (2016) Applications of structural equation modeling (SEM) in ecological studies: an updated review. Ecol Process 5(1): 19. https://doi.org/10.1186/s13717-016-0063-3

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Funct Plant Biol 11(6): 539-552. https://doi.org/10.1071/PP9840539

Forrester DI, Collopy JJ, Beadle CL, Baker TG (2012) Interactive effects of simultaneously applied thinning, pruning and fertiliser application treatments on growth, biomass production and crown architecture in a young *Eucalyptus nitens* plantation. For Ecol Manag 267: 104-116. https://doi.org/10.1016/j.foreco.2011.11.039

Ge Z, Man X, Cai T, Duan B, Xiao R, Xu Z (2022) Environmental factors at different canopy heights had measurable effects on leaf physiology. Sustainability 14(9): 5126. https://doi.org/10.3390/su14095126

González-Armas R, Vilà-Guerau de Arellano J, Mangan MR, Hartogensis O, De Boer H (2024) Impact of canopy environmental variables on the diurnal dynamics of water and carbon dioxide exchange at leaf and canopy level. Biogeosciences 21: 2425-2445. https://doi.org/10.5194/bg-21-2425-2024

Hatfield JL, Dold C (2019) Water-use efficiency: advances and challenges in a changing climate. Front Plant Sci 10: 103. https://doi.org/10.3389/fpls.2019.00103

Hu L, Bentler PM (1999) Cut off criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Struct Equ Model 6: 1–55. https://doi.org/10.1080/10705519909540118

Jaimes-Suárez YY, Carvajal-Rivera AS, Galvis-Neira DA, Carvalho FE, Rojas-Molina J (2022) Cacao agroforestry systems beyond the stigmas: Biotic and abiotic stress incidence impact. Front Plant Sci 13: 921469. https://doi.org/10.3389/fpls.2022.921469

Keprate A, Bhardwaj DR, Sharma P, Verma K, Abbas G, Sharma V, Janju S (2024) Climate resilient agroforestry systems for sustainable land use and livelihood. In: Kanga S, Singh SK, Shevkani K, Pathak V, Sajan B (ed) Transforming Agricultural Management for a Sustainable Future. World Sustainability Series. Springer. pp 325 https://doi.org/10.1007/978-3-031-63430-7_7

Killick R, Eckley IA (2014) Changepoint: An R Package for Changepoint Analysis. J Stat Softw 58(3): 1–19. https://doi.org/10.18637/jss.v058.i03

Kline RB (2023) Principles and practice of structural equation modeling. Guilford publications. New York. Pp 196

Lamour J, Davidson KJ, Ely KS, Le Moguédec G, Anderson JA, Li Q, Calderón O, Koven CD, Wright SJ, Walker AP, Serbin SP Rogers A (2023) The effect of the vertical gradients of photosynthetic parameters on the CO2 assimilation and transpiration of a Panamanian tropical forest. New Phytol 238(6): 2345-2362. https://doi.org/10.1111/nph.18901

Lahive F, Hadley P, Daymond AJ (2019) The physiological responses of cacao to the environment and the implications for climate change resilience. A review. Agron Sustain Dev 39(1): 5. https://doi.org/10.1007/s13593-018-0552-0

586 Laurans M, Hérault B, Vieilledent G, Vincent G (2014) Vertical stratification reduces competition for light
587 in dense tropical forests. For Ecol Manag 329: 79-88. http://dx.doi.org/10.1016/j.foreco.2014.05.059

588 Lebrija-Trejos E, Pérez-García EA, Meave JA, Bongers F, Poorter L (2010) Functional traits and
589 environmental filtering drive community assembly in a species-rich tropical system. Ecology 91(2): 386-
590 398. https://doi.org/10.1890/08-1449.1

591 Li W, Li B, Ma X, Saha S, Wu H, Zhang P, Shen H (2023) Physiological and biochemical traits of needles
592 imply that understory light conditions in the growing season may be favorable to *Pinus koraiensis*
593 Trees. Forests 14(7): 1333. https://doi.org/10.3390/f14071333

594 Lin BB (2010) The role of agroforestry in reducing water loss through soil evaporation and crop
595 transpiration in coffee agroecosystems. Agric For Meteorol 150(4): 510-518.
596 https://doi.org/10.1016/j.agrformet.2009.11.010

597 Lowman M (2021) Life in the treetops—An overview of forest canopy science and its future
598 directions. Plants People Planet 3(1): 16-21. https://doi.org/10.1002/ppp3.10125

599 Lowman MD, Schowalter TD (2012) Plant science in forest canopies–the first 30 years of advances and
600 challenges (1980–2010). New Phytol 194(1): 12-27. https://doi.org/10.1111/j.1469-8137.2012.04076.x

601 Maurin V, DesRochers A (2013) Physiological and growth responses to pruning season and intensity of
602 hybrid poplar. For Ecol Manag 304: 399-406. https://doi.org/10.1016/j.foreco.2013.05.039

603 Maydeu-Olivares A (2017) Maximum likelihood estimation of structural equation models for continuous
604 data: Standard errors and goodness of fit. Struct Equ Model 24(3): 383-394.
605 https://doi.org/10.1080/10705511.2016.1269606

606 Medrano H, Tomás M, Martorell S, Flexas J, Hernández E, Rosselló J, Pou A, Escalona JM, Bota J (2015)
607 From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a
608 selection target. The Crop Journal 3(3): 220-228. https://doi.org/10.1016/j.cj.2015.04.002

609 Meinzer FC, Campanello PI, Domec JC, Gatti MG, Goldstein G, Villalobos-Vega R, Woodruff DR (2008)
610 Constraints on physiological function associated with branch architecture and wood density in tropical
611 forest trees. Tree Physiol 28(11): 1609-1617. https://doi.org/10.1093/treephys/28.11.1609

612 Miyaji KI, Da Silva WS, Alvim PDT (1997) Longevity of leaves of a tropical tree, *Theobroma cacao*, grown
613 under shading, in relation to position within the canopy and time of emergence. New Phytol 135(3):
614 445-454. https://doi.org/10.1046/j.1469-8137.1997.00667.x

615 Mulkey SS, Kitajima K, Wright SJ (1996) Plant physiological ecology of tropical forest canopies. Trends
616 Ecol Evol 11(10): 408-412. https://doi.org/10.1016/0169-5347(96)10043-4

617 Niether W, Jacobi J, Blaser WJ, Andres C, Armengot L (2020) Cocoa agroforestry systems versus
618 monocultures: a multi-dimensional meta-analysis. Environ Res Lett 15(10): 104085.
619 https://doi.org/10.1088/1748-9326/abb053

620 Ntawuhiganayo EB, Uwizeye FK, Zibera E, Dusenge ME, Ziegler C, Ntirugulirwa B, Nsabimasa D, Wallin G,
621 Uddling J (2020) Traits controlling shade tolerance in tropical montane trees. Tree Physiol 40(2): 183-
622 197. https://doi.org/10.1093/treephys/tpz119

623 Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell
624 WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L,
625 Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter
626 Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino

627 S, Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits
628 worldwide. Aust J Bot 61: 167–234. https://doi.org/10.1071/BT12225

629 Pipitsunthonsan P, Sopharat J, Sirisuk P, Chongcheawchamnan M (2018) Leaf sensor for stomata
630 transpiration monitoring using temperature and humidity. In: Chiang R (ed) 21st International
631 Symposium on Wireless Personal Multimedia Communications (WPMC). Thailand. pp. 252-255.
632 https://doi.org 10.1109/WPMC.2018.8713096.

633 Poorter L, Bongers F (2006) Leaf traits are good predictors of plant performance across 53 rain forest
634 species. Ecology 87(7): 1733-1743. https://doi.org/10.1890/0012-9658(2006)87[1733:LTAGPO]2.0.CO;2

635 Poorter L (2007) Are species adapted to their regeneration niche, adult niche, or both? Am Nat 169(4):
636 433-442. https://doi.org/10.1086/512045

637 R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical
638 Computing, Vienna, Austria. https://www.R-project.org/

639 Ray T, Fichtner A, Kunz M, Proß T, Bradler PM, Bruelheide H, Georgi l, Haider S, Hildebrand M, Potvin C,
640 Schnabel F, Trogisch S, von Oheimb G (2024) Diversity-enhanced canopy space occupation and leaf
641 functional diversity jointly promote overyielding in tropical tree communities. Sci Total Environ 951:
642 175438. https://doi.org/10.1016/j.scitotenv.2024.175438

643 Reich PB, Flores-Moreno H (2017) Peeking beneath the hood of the leaf economics spectrum. New
644 Phytol 214(4): 1395-1397. https://www.jstor.org/stable/90010379

645 Read J, Stokes A (2006) Plant biomechanics in an ecological context. Am J Bot 93(10): 1546–1565.
646 https://doi.org/10.3732/ajb.93.10.1546

Rosseel Y (2012) lavaan: An R Package for Structural Equation Modeling. J Stat Softw 48(2): 1–36. https://doi.org/10.18637/jss.v048.i02

Rozendaal DMA, Hurtado VH, Poorter L (2006) Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. Funct Ecol 20(2): 207-216. https://www.jstor.org/stable/3806552

Rüegg J, Saj S, Schneidewind U, Milz J, Schneider M, Armengot L (2025) Competitive yields in organic and agroforestry cacao cropping systems: Results from 15 years of a long-term systems comparison trial in Bolivia. Agron Sustain Dev 45(6): 76. https://doi.org/10.1007/s13593-025-01073-8

Saavedra F, Jordan Peña E, Schneider M, Naoki K (2020) Effects of environmental variables and foliar traits on the transpiration rate of cocoa (*Theobroma cacao* L.) under different cultivation systems. Agrofor Syst 94: 2021–2031. https://doi.org/10.1007/s10457-020-00522-5

Saj S, Somarriba E, Schneidewind U, Milz J, Cotter M, Schneider M (2025) Shade tree pruning effects on flowering and cacao yields in different cropping systems in a long-term trial in Bolivia. Exp Agric 61: e22. https://doi.org/10.1017/S0014479725100124

Schielzeth H (2010) Simple means to improve the interpretability of regression coefficients. Methods Ecol Evol 1(2): 103-113. https://doi.org/10.1111/j.2041-210X.2010.00012.x

Somarriba E, Orozco-Aguilar L (2018) Analysis and design of the shade canopy of cocoa-based agroforestry systems. In: Umaharan, P. (ed.), Achieving sustainable cultivation of cocoa, Burleigh Dodds Science Publishing, Cambridge, UK. pp 876.

666 Sommer B, Harrison PL, Beger M, Pandolfi JM (2014) Trait-mediated environmental filtering drives
667 assembly at biogeographic transition zones. Ecology 95(4): 1000-1009. https://doi.org/10.1890/13-
668 1445.1

669 Sun T, Cheng R, Jiang R, Liu Y, Sun Y, Wang Z, Fang P, Wu X, Ning K, Xu P (2023) Combining functional
670 physiological phenotyping and simulation model to estimate dynamic water use efficiency and infer
671 transpiration sensitivity traits. Eur J Agron 150 : 126955. https://doi.org/10.1016/j.eja.2023.126955

672 Symonds MR, Moussalli A (2011) A brief guide to model selection, multimodel inference and model
673 averaging in behavioural ecology using Akaike's information criterion. Behav Ecol Sociobiol 65(1): 13-21.
674 https://doi.org/10.1007/s00265-010-1037-6

675 Torrez V, Jørgensen PM, Zanne AE (2013) Specific leaf area: a predictive model using dried samples. Aust
676 J Bot 61(5): 350-357. https://doi.org/10.1071/BT12236

677 Vinod N, Slot M, McGregor IR, Ordway EM, Smith MN, Taylor TC, Sack L, Buckley TN, Anderson-Teixeira
678 KJ (2023) Thermal sensitivity across forest vertical profiles: patterns, mechanisms, and ecological
679 implications. New Phytol 237(1): 22-47. https://doi.org/10.1111/nph.18539

680 Walter H (1939) Ökologische Pflanzengeographie. In Band A (ed) Fortschritte der Botanik (pp. 241-260).
681 Berlin, Heidelberg: Springer Berlin Heidelberg. pp 260.

682 Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T,
683 Cornelissen JHC, Matthias D, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee
684 T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L,
685 Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf
686 economics spectrum. Nature, 428(6985): 821-827. https://doi.org/10.1038/nature02403

687 Wu Q, Wang L (2024) Farmland hydrological cycle under agroforestry systems and efficient use of water
688 resources in the karst desertification environment. Heliyon 10(15): e35506.
689 https://doi.org/10.1016/j.heliyon.2024.e35506

690 Xing S, Leahy L, Ashton LA, Kitching RL, Bonebrake TC. Scheffers BR (2023) Ecological patterns and
691 processes in the vertical dimension of terrestrial ecosystems. J Anim Ecol 92(3): 538-551.
692 https://doi.org/10.1111/1365-2656.13950

693 Yu F, Gara TW, Lian J, Ye W, Shen J, Wang T, Wu Z, Wang J (2021) Understanding the impact of vertical
694 canopy position on leaf spectra and traits in an evergreen broadleaved forest. Remote Sens 13(24):
695 5057. https://doi.org/10.3390/rs13245057

696 Zheng LT, He D, He XM, Yin F, Yan ER (2025) Neighborhood interactions and environment modulate
697 individual-level trait correlations and divergent functional trade-offs among co-occurring trees. J Plant
698 Ecol 18(6): rtaf099. https://doi.org/10.1093/jpe/rtaf099

Tables

Table 1 Standardised factor loadings and significance levels (p-values) of observed variables for the three structural equation models. Bold values indicate statistically significant loadings (***: p < 0.001; **: p < 0.01; *: p < 0.05; °: p <0.07). WUE_{inst} = Instantaneous water-use efficiency, *A* = CO_2 assimilation rate, E = Transpiration rate, PAR = Photosynthetically active radiation, Str = Vertical strata, LA = Leaf area, SLA = Specific leaf area, RWC = Relative water content, SD = Stomatal density, Tmp = Air temperature and Hmd = Relative air humidity (Conceptual model in Figure 1).

Latent variable	Observed indicator	WUE_{inst}		*A*		E	
Vegetation Structure (Vst)		**0.309**	***	**-0.480**	***	**0.174**	***
	PAR	**0.707**	***	**0.707**	***	**0.707**	***
	Str	**0.826**	***	**0.850**	***	**0.799**	***
	Env	0.004		**0.546**	**	0.080	
Traits (Trt)		**0.129**	***	**-0.650**	***	**-0.510**	***
	RWC	**0.707**	***	**0.487**	***	**0.707**	***
	LA	0.530		**0.489**	***	0.551	
	SLA	-0.075		**-0.177**	***	-0.010	
	SD	0.021		-0.028		0.024	
	Env	-0.042		**-0.666**	**	-0.120	°
Microenvironment (Env)	Tmp	**0.707**	***	**0.453**	***	**0.709**	***
	Hmd	**-0.454**	***	**-0.480**	***	**-0.460**	***

Table 2 Comparison of best linear models for Instantaneous water use efficiency (WUE_{inst}) by vertical strata. Hmd = Relative air humidity, PAR = Photosynthetically active radiation, LA = Leaf area, SLA = Specific leaf area and RWC = Relative water content. Significant t-values are followed by ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

	Estimate	Std. Error	D.f.	t-value	
WUE_{inst} – Low stratum (R^2 marginal = 0.19)					
Intercept	-0.361	0.104	15.93	-3.439	**
Hmd	0.268	0.044	231.2	6.031	***
PAR	0.293	0.080	132.6	3.647	***
SLA	0.306	0.078	224.12	3.929	***
WUE_{inst} – Middle stratum (R^2 marginal = 0.06)					
Intercept	-0.247	0.084	120	-2.937	**
Hmd	0.193	0.091	120	2.121	*
PAR	0.269	0.110	120	2.438	*
WUE_{inst} – High stratum (R^2 marginal = 0.19)					
Intercept	0.041	0.174	14.15	0.241	
PAR	0.241	0.098	110.36	2.455	*
LA	0.616	0.162	63.3	3.797	***
SLA	-0.250	0.093	124.11	-2.685	**
RWC	-0.294	0.149	124.83	-1.969	*
WUE_{inst} – Emergent stratum (R^2 marginal = 0.08)					
Intercept	0.857	0.105	64	8.146	***

Table 3 Comparison of linear models for CO_2 assimilation rate (*A*) by vertical strata. Hmd = Relative air humidity, Tmp = Air temperature, PAR = Photosynthetically active radiation, LA = Leaf area, SLA = Specific leaf area and SD = Stomata density. Significant t-values are followed by ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$.

	Estimate	Std. Error.	D.f.	t-value	
***A* - Low stratum (R^2 marginal = 0.21)**					
Intercept	-0.151	0.103	8.74	-1.463	
PAR	0.336	0.062	138.84	5.349	***
LA	-0.429	0.104	99.89	-4.113	***
SD	0.200	0.079	25.60	2.534	*
***A* - Middle stratum (R^2 marginal = 0.35)**					
Intercept	-0.224	0.166	12.33	-1.347	
Hmd	-0.207	0.079	113.03	-2.598	*
LA	-0.389	0.125	34.92	-3.100	**
SLA	-0.245	0.098	61.57	-2.499	*
***A* - High stratum (R^2 marginal = 0.40)**					
Intercept	-0.133	0.409	8.65	-0.327	
Tmp	0.138	0.065	113.13	2.098	*
LA	-1.138	0.165	101.78	-6.884	***
SD	-0.281	0.143	124.85	-1.964	*
***A* – Emergent stratum (R^2 marginal = 0.53)**					
Intercept	0.943	0.155	64	6.051	***
LA	-0.980	0.116	64	-8.446	***

Table 4 Comparison of linear mixed models for Transpiration rate (E) by vertical strata. Hmd = Relative air humidity, PAR = Photosynthetically active radiation, LA = Leaf area and SLA = Specific leaf area. Significant t-values are followed by ***: p < 0.001; **: p < 0.01; *: p < 0.05.

	Estimate	Std. Error	D.f.	t-value	
E – Low stratum (R^2 marginal = 0.34)					
Intercept	-0.094	0.182	10.51	-0.517	
Hmd	-0.201	0.029	227.71	-6.947	***
PAR	0.157	0.057	235.83	2.742	**
LA	-0.823	0.102	169.03	-8.004	***
E – Middle stratum (R^2 marginal = 0.53)					
Intercept	-0.065	0.144	15.95	-0.456	
Hmd	-0.300	0.079	115.35	-3.776	***
LA	-0.608	0.115	34.87	-5.285	***
SLA	-0.220	0.092	65.79	-2.378	*
E – High stratum (R^2 marginal = 0.53)					
Intercept	-0.198	0.318	9.22	-0.622	
Hmd	-0.159	0.043	113.1	-3.687	***
LA	-1.165	0.121	111.41	-9.579	***
E – Emergent stratum (R^2 marginal = 0.64)					
Intercept	0.296	0.116	64	2.544	*
LA	-0.936	0.086	64	-10.791	***

Figures

Fig1.jpg. Schematic representation of the effects of the microenvironment (Env), Leaf traits (Trt) and Vegetation structure (Vst) on Instantaneous water-use efficiency (WUE_{inst}), CO_2 assimilation rate (*A*), and Transpiration rate (E) across four canopy strata. Hmd = Relative air humidity, Tmp = Air temperature, PAR = Photosynthetically Active Radiation, Str = Vertical strata, LA = Leaf area, SLA = Specific leaf area, RWC = Relative water content and SD = Stomata density.

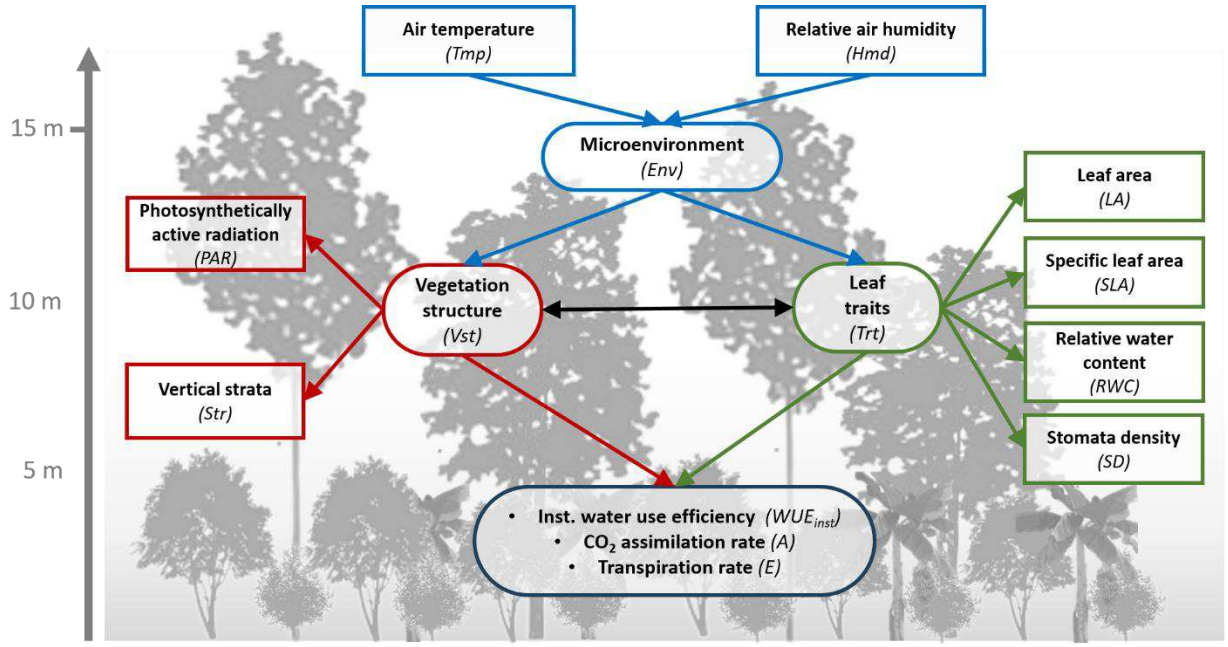
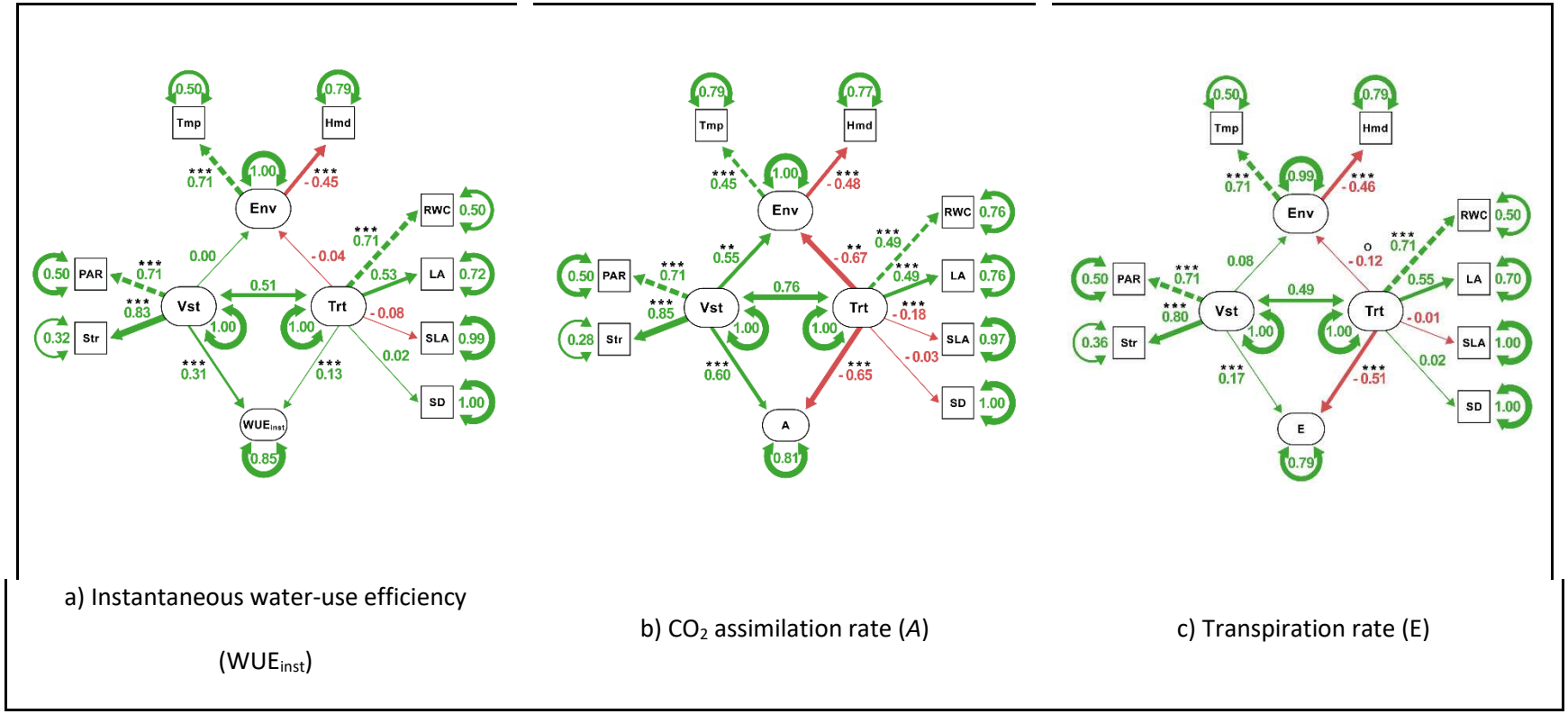
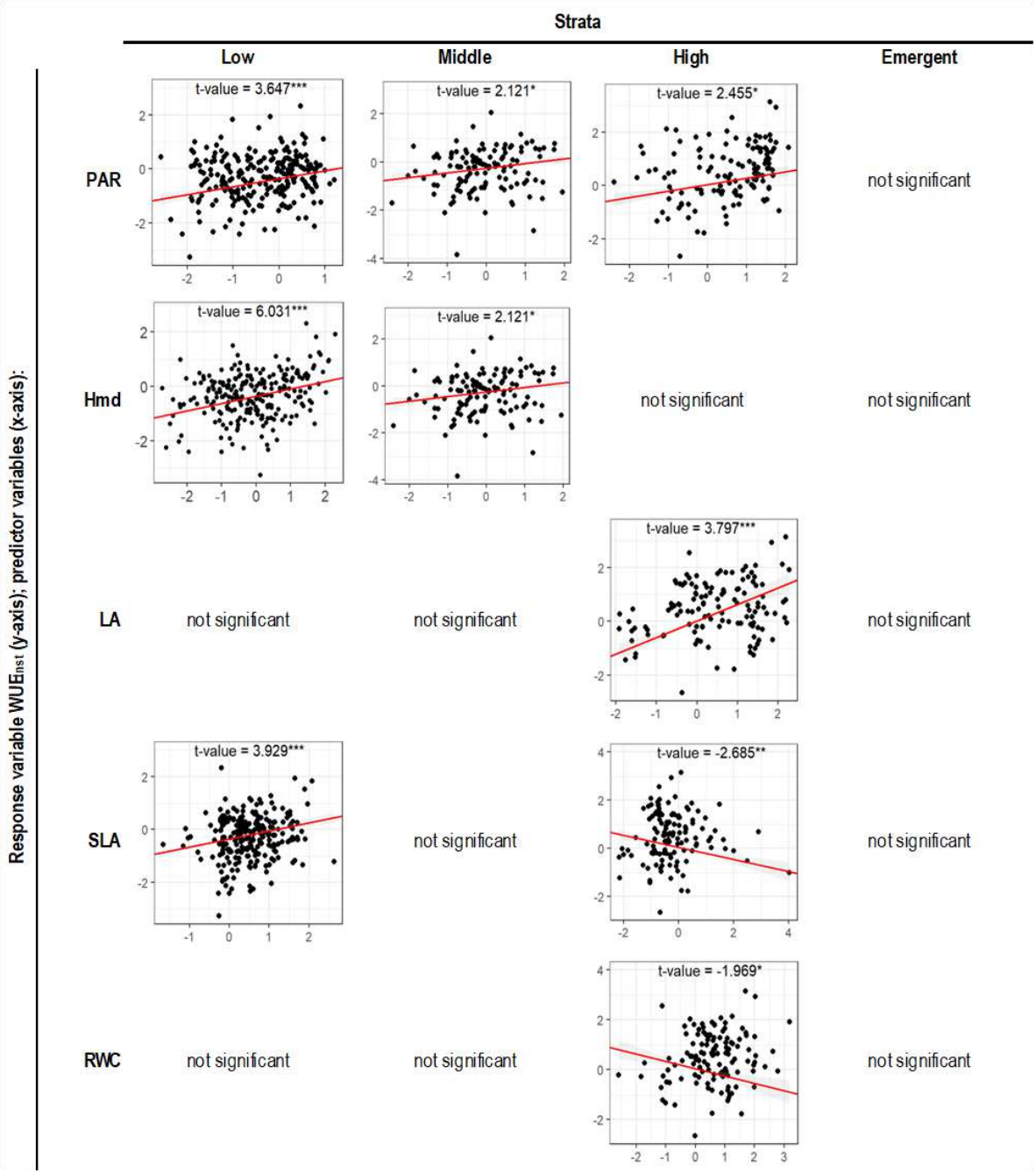


Fig2.eps. Comparison of direct and indirect effects of Microenvironmental variables (Env), Vegetation structure (Vst) and Leaf traits (Trt) on: a) Instantaneous water-use efficiency (WUE_{inst}), b) CO_2 assimilation rate (*A*) and c) Transpiration rate (E). Bold full lines show significant relationships, dashed lines show the intercepts in the models, non-bold lines represent non-significant variables. Green represents positive effects and red negative ones. Significant (***: p < 0.001; **: p < 0.01; *: p < 0.05).



738 **Fig3.tiff.** Linear relationships between PAR = Photosynthetically active radiation, Hmd = Relative air
739 humidity, LA = Leaf area, SLA = Specific leaf area, and RWC = Relative water content (as predictor
740 variables) with Instantaneous water-use efficiency (WUE_{inst}) (as response variable) across the four
741 vertical strata. Significant (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$).



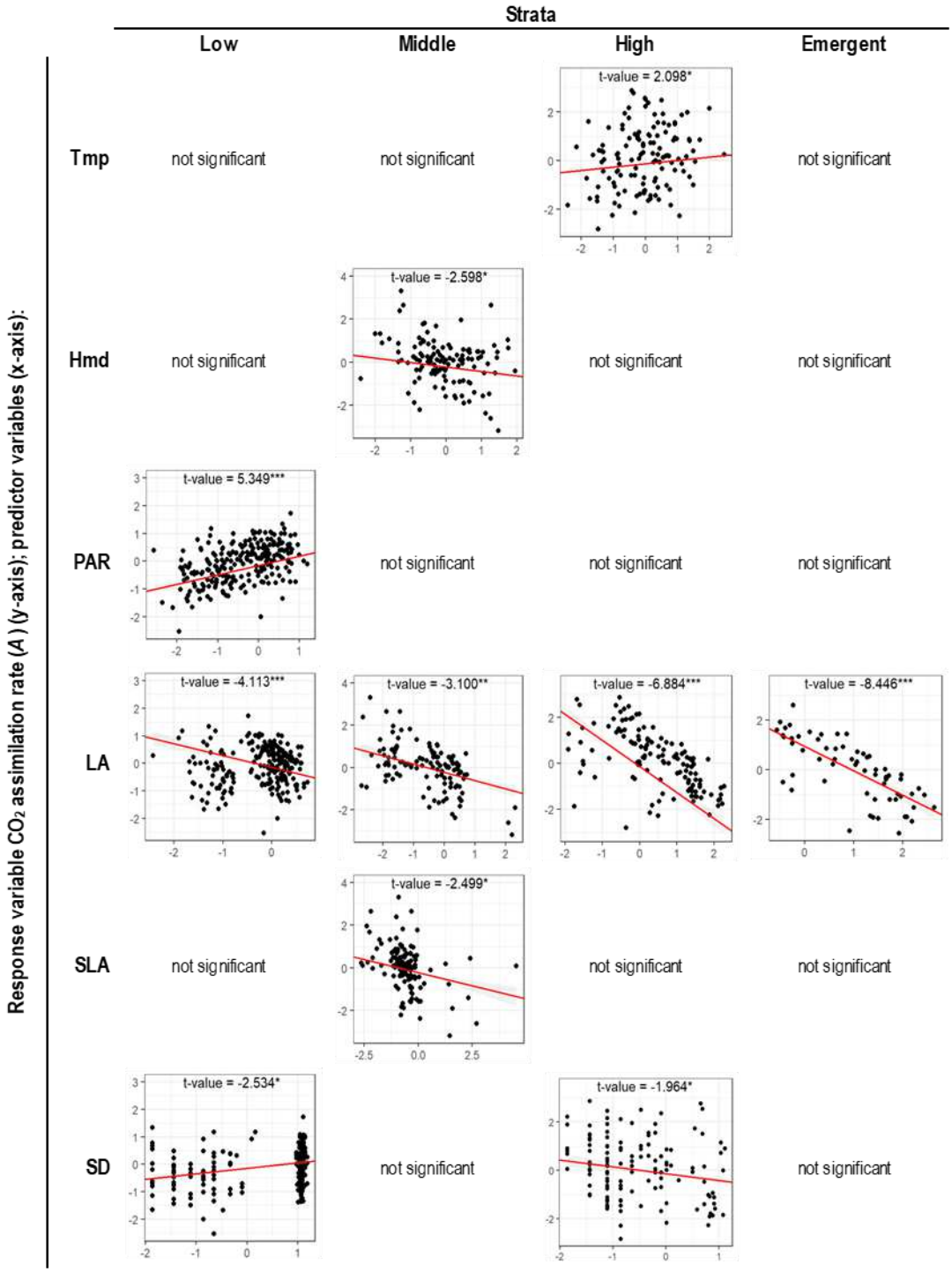
742

743 **Fig4.tiff.** Linear relationships between Tmp = Air temperature, Hmd = Relative air humidity, PAR =

744 Photosynthetically active radiation, LA = Leaf area, SLA = Specific leaf area and SD = Stomata density (as

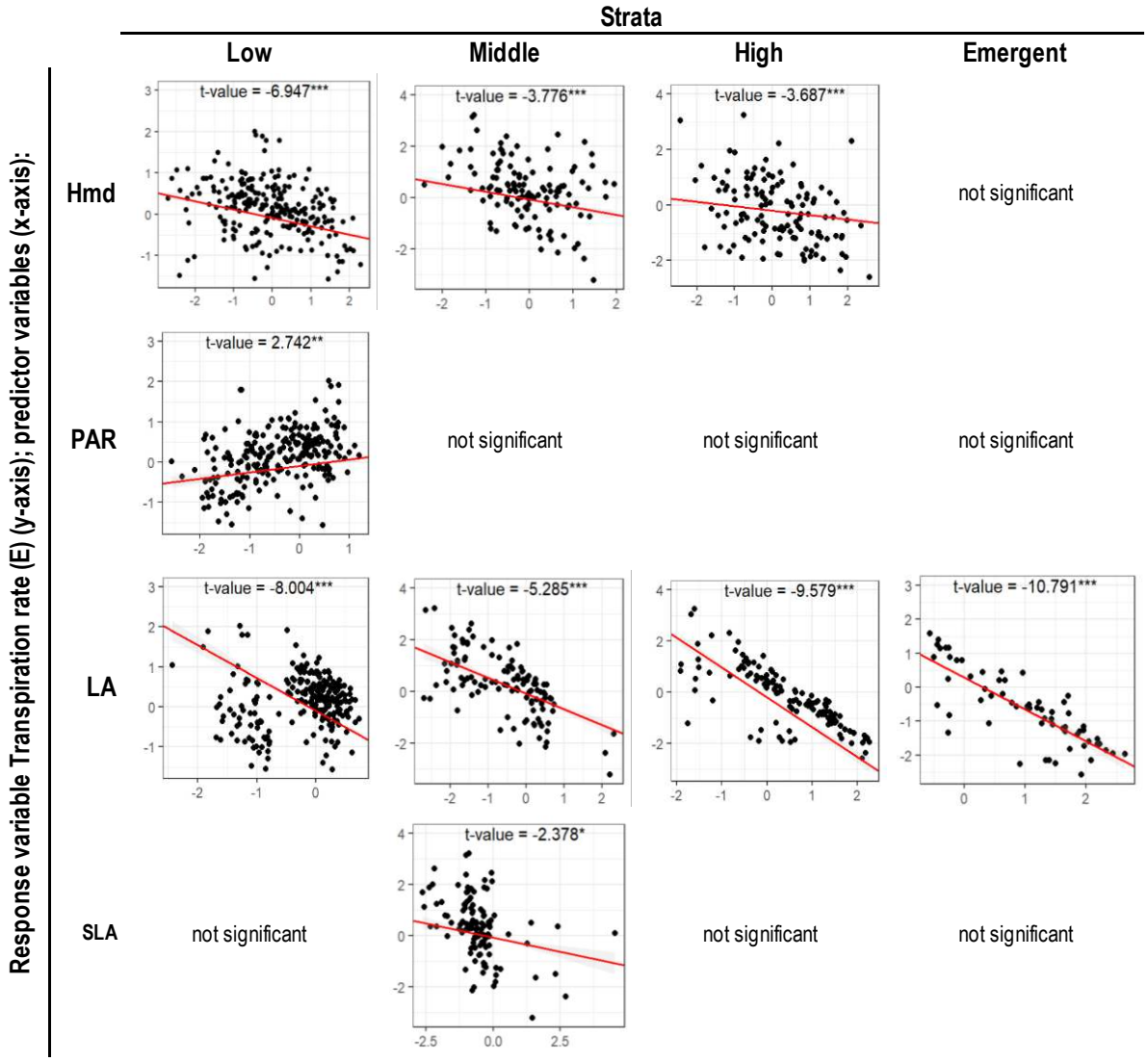
745 predictor variables) with CO_2 assimilation rate (as response variable) across the four vertical strata.

746 Significant (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$).



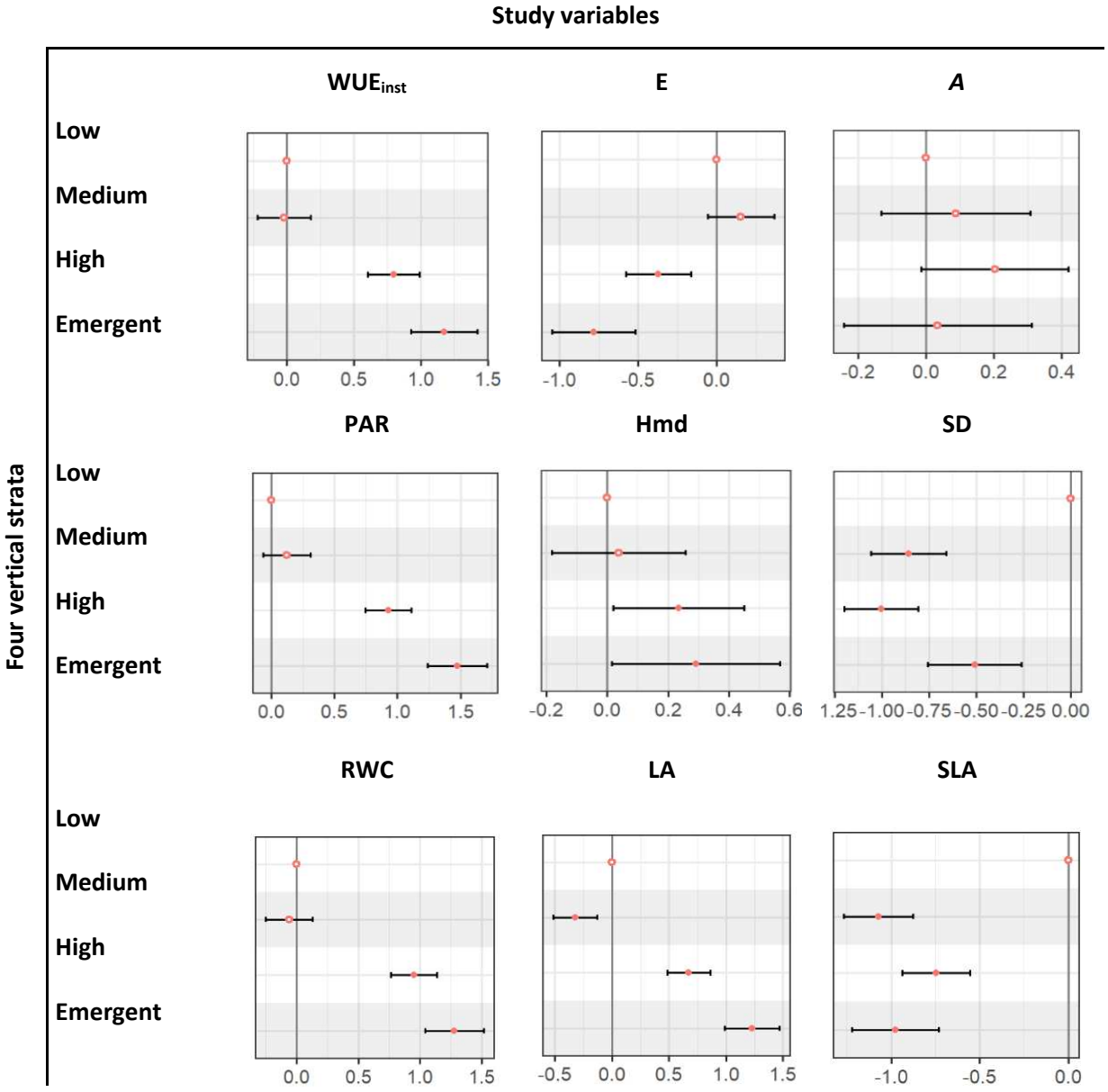
747

748 **Fig5.tiff.** Linear relationships between Hmd = Relative air humidity, PAR = Photosynthetically active
749 radiation, LA = Leaf area, and SLA = Specific leaf area (as predictor variables) with Transpiration rate (as
750 response variable) across the four vertical strata studied. Significant (***: p < 0.001; **: p < 0.01; *: p <
751 0.05).



752

Fig6.tiff. Beta coefficients from Linear Models (LM) of physiological, microenvironmental, and leaf trait variables compared across the four vertical strata. WUE_{inst} = Water use efficiency, E = Transpiration rate, *A* = CO_2 assimilation rate, PAR = Photosynthetically active radiation, Hmd = Relative air humidity, SD = Stomatal density, RWC = Relative water content, LA = Leaf area and SLA = Specific leaf area.



- Total number of words (including references): 9538
- Total number of words (excluding references): 7494
- Abstract number of words: 250
- Number of words in Supplementary Information: 1319

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarymaterialFinal.docx