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Effects of environmental variables and foliar traits on the transpiration rate of cocoa (*Theobroma cacao* L.) under different cultivation systems

Francisco Saavedra · Ernesto Jordan Peña · Monika Schneider · Kazuya Naoki

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Abstract The response of plant species to environmental conditions influences changes in functional traits associated with the process that determines biological fitness and ecosystem processes. However, documenting these responses remain largely elusive in cultivation systems. We analyzed how environmental variables and leaf traits have effects on the transpiration rate of cocoa (*Theobroma cacao* L.) trees compared among different cultivation systems. Fieldwork was carried out at the Sara Ana experimental station in Alto Beni, La Paz, Bolivia. We sampled four trees in each of eight plots; four plots for each cultivation system (organic monoculture vs. organic agroforestry). From each tree, two mature, sunlit and healthy leaves were collected to make measurements of foliar traits and environmental variables. We found that canopy cover was higher in the agroforestry system. The specific leaf area was greater in agroforestry but the stomata size was significantly higher in the monoculture system. Temperature had a positive relationship with transpiration, whereas canopy cover and specific leaf area had a negative relationship in the agroforestry system. Cultivation system caused changes in microenvironmental conditions and on the expression of leaf traits that regulate water flow through the plant. Thus, the mutual effects of canopy cover, larger leaves and smaller stomatal size may drive a more efficient water use by reducing the transpiration rate of plants growing in agroforestry systems.

Keywords Agroforestry · Canopy cover · Specific leaf area · Stomata size · Temperature

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F. Saavedra (✉)
Herbario Nacional de Bolivia, Instituto de Ecología, Universidad Mayor de San Andrés, Campus universitario Cota Cota c/27, La Paz, Bolivia
e-mail: fsaavedraa@fcpn.edu.bo

E. Jordan Peña
Carrera de Biología, Facultad de Ciencias Puras y Naturales, Universidad Mayor de San Andrés, Campus universitario Cota Cota c/27, La Paz, Bolivia

M. Schneider
Department of International Cooperation, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, 5070 Frick, Switzerland

K. Naoki
Instituto de Ecología, Carrera de Biología, Universidad Mayor de San Andrés, Campus universitario Cota Cota c/27, La Paz, Bolivia

Introduction

The responses of plant species to environmental conditions influence changes in functional traits associated with physiological (Monteiro et al. 2016) and ecosystem processes (Nock et al. 2016). Functional traits comprise a variety of features that influence the life strategies of organisms (Pérez-Harguindeguy et al. 2016). Consequently, the variation of functional traits will be associated with the gradient of variation in environmental conditions (Garnier et al. 2016).

The interaction between environmental conditions and leaf traits can be used to quantify the effects on physiological processes such as transpiration (Garnier et al. 2016; Monteiro et al. 2016). Among the leaf traits with greater responses to changes in environmental conditions, are the specific leaf area (SLA), stomatal density (SD) and stomatal occlusive cell size (SZ) (Wright et al. 2004). The SLA is a functional trait used to describe the morpho-anatomical structure of leaves. Several studies have found a correlation of SLA with leaf respiration, maximum photosynthesis, relative growth, leaf nitrogen content and water use efficiency at the species and community level (Daymond et al. 2011; Poorter and Bongers 2006), and is considered a good predictor of the performance and life strategy of plants (Garnier et al. 2016). Physiological traits such as transpiration will respond to variations of morfo-anatomical leaf traits such as SLA, SD and SZ (Monteiro et al. 2016) and hydrological traits such as leaf relative water content (LRWC), and vapor pressure deficit (VPD). The transpiration of water vapor fulfills several metabolic and physiological functions, among which it allows the circulation of water and transport of nutrients through the plants' vascular system (Hölttä et al. 2006), avoiding overheating of leaves by heat loss thought evaporation (Monteiro et al. 2016; Rozendaal et al. 2006) and contributes to the carbon regulation and water cycles (Taylor et al. 2012).

Different cultivation systems have physiological effects on the leaf (Bote and Struik 2011; Lin 2010). For example, an increase in solar radiation will increase the rate of photosynthesis, but also transpiration and water requirements of the plant (Daymond et al. 2011). Therefore, it should be expected that the environmental conditions in different cultivation systems have effects on leaf traits and physiological processes of the cultivated species (Balasimha et al. 1991). Cocoa has certain functional plasticity that allows it to adapt to different environmental conditions (Avila-Lovera et al. 2015). However, until now which leaf traits drive changes in leaf transpiration and how much of the variation is due to environmental conditions generated in different cultivation systems has not been tested.

The present study aims to analyze how environmental conditions and leaf traits effect on the transpiration rate among different cocoa cultivation systems (i.e., monoculture and agroforestry). In this context, we asked: How do cultivation systems influence changes in environmental variables and leaf traits? and How do leaf traits and environmental variables influence the transpiration of cocoa trees under different cultivation systems? According to Smith and Huston (1990) plants that are shade-tolerant are not tolerant to drought, that is a compromise between tolerance to shade or to drought. Under this theory, a first hypothesis is that humidity is not a limiting factor, so trees that receive more sunlight are more active physiologically, and it does not matter whether they are in environments with lower humidity (Balasimha et al. 1991). We predict that under monoculture systems, cocoa trees will display leaf traits that will increase the rate of photosynthesis, such as high stomatal density, large stomata size, greater leaf area and high transpiration. An alternative hypothesis is that humidity is a limiting factor, so trees with less vegetative cover suffer more water stress and will develop functional traits that avoid water loss and adapt their morphology to environments with lower humidity (Lahive et al. 2019). We predict that under monocultures, cocoa trees will display leaf traits to avoid water loss, such as low stomatal density, small stomata size, lower leaf area and lower transpiration. A third hypothesis is that there is no such compromise and that tolerance to shade is uncoupled from tolerance to drought or are coordinated characteristics (Markesteijn et al. 2011). To test these hypotheses, we measured different leaf traits, leaf transpiration and environmental variables in plots under different cocoa cultivation systems.

Materials and methods

Study site description

Fieldwork was carried out in November 2017, at Sara Ana, Alto Beni, La Paz department, Bolivia (15° 27′ 36″ S–67° 28′ 17″ W, 450 m a.s.l.). The mean annual temperature is 25.2 ± 0.5 °C, and annual precipitation is 1441 ± 243 mm at Sapecho, a nearby town located 20 km SE of Sara Ana (Schneider et al. 2016). In 2008 a long-term trial of different cocoa cultivation systems was established in a completely randomized block design with four repetitions of each treatment (Armengot et al. 2016). The site was divided in 24 plots (48 × 48 m, 2304 m^2), which were assigned into four groups of six plots each. In each group, one of the six treatments was randomly assigned: (1) fallow (FA), (2) successional agroforestry (SA), (3) organic agroforestry (AO), (4) conventional agroforestry (AC), (5) shade-less organic monoculture (MO), and (6) shade-less conventional monoculture (MC) (Fig. 1).

Of all these cultivation systems, we selected: organic monoculture (MO) and organic agroforestry (AO) to conduct our data collection because most cacao farms in Bolivia use organic systems. MO have a simple structure with few or no accompanying species, where cocoa grows without shade (Rice and Greenberg 2000; Almeida and Valle 2007), or chemical fertilizers or pesticides (Schneider et al. 2016). AO differs from MO in the presence of a complex structure and diversity of companion trees in a polyculture system with shade (Rice and Greenberg 2000). The shade providing species are banana (*Musa* spp.), legumes trees such as *Inga* spp. and *Erythrina* spp., and timber and fruit tree species at a lower density like mahogany (*Swietenia macrophylla*) and avocado (*Persea americana*) (Schneider et al. 2016).

Study species

Two cocoa (*Theobroma cacao* L., Malvaceae) varieties (IIa 22 and TSH 565) were selected as study species. IIa 22, is a local variety identified by producers and technicians in an elite cocoa tree selection program in Alto Beni, Bolivia (Trujillo 2007; Schneider et al. 2016). THS 565 is a “Trinitario” variety, and information about its origin, characteristics and germplasm is documented in the International Cocoa Germplasm Database (ICGD) (Turnbull and Hadley 2014). According to Yang et al. (2013) TSH 565 variety is more related to “Forastero” lineage from South America. Thus, since both varieties have the same geographical origin they may be more related to “Forastero” lineage (Motamayor et al. 2008).

Experimental design

Four trees were selected from each of the two studied varieties in two organic cocoa cultivation systems (i.e., MO, AO). From each tree, two mature (same age and position), healthy leaves, without traces of herbivory and directly exposed to solar radiation, were collected to measure foliar traits, long-term and short-term environmental variables. Sampling was replicated in eight plots, four plots for each type of cultivation system. Long and short-term environmental variables were measured on the same day in both cultivation systems to control the variability in cloudiness that can occur from 1 day to another. For statistical analysis we pooled data of both cocoa varieties because no differences were found between them, may be due to similar geographical origin and lineage. Thus, in total, the leaf traits of 112 leaves of 56 trees belonging to two varieties of cocoa were measured in two cocoa cultivation systems.

Long and short-term environmental variables

Since all the plots are exposed to the same climatic conditions, it is expected that the microenvironmental variation among trees is due to the influence of neighboring trees and plants. This is why the availability of light and water were measured as canopy cover. For light availability, we used the crown exposure index (CE) of Dawkins and Fields (1978), which is a qualitative measure of ligth exposure around and above the canopy of a tree. This index is classified on five categories: 1 = no direct exposure, 2 = lateral exposure, 3 = partial above exposure, 4 = more than 90% of above direct exposure, and 5 = emergent crowns exposed to light in all directions (Poorter et al. 2005). It has the advantage that it is a more general measure than the direct measurement of the photon flux or the photosynthetically active radiation, which only give instantaneous information of the light condition (Keeling and Phillips 2007).

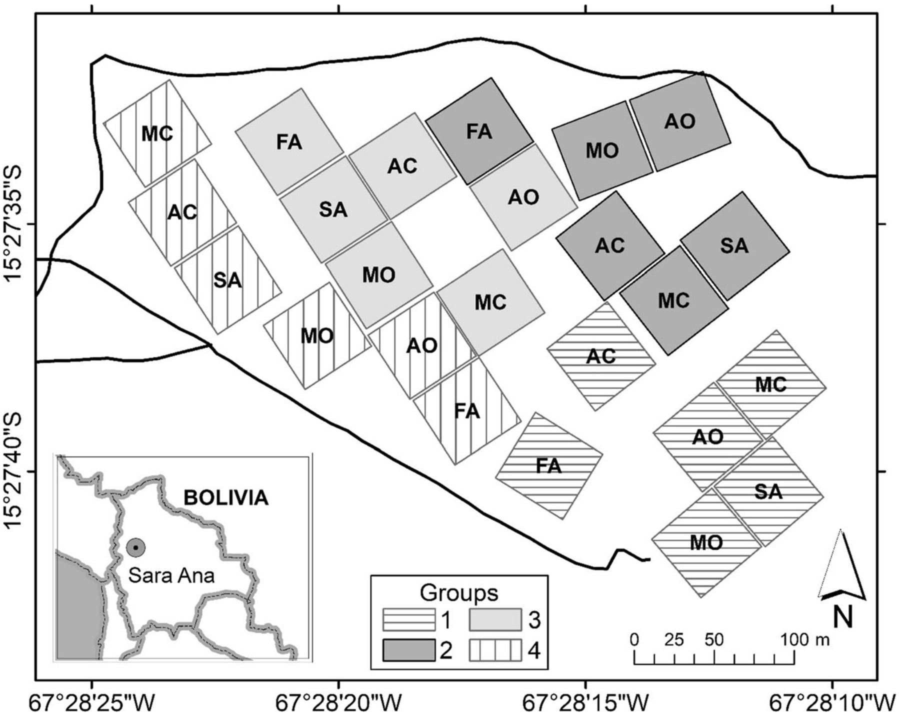

Fig. 1 Map of the long-term trial site in Sara Ana, La Paz, Bolivia. FA: fallow, SA: successional agroforestry, AO: organic agroforestry, AC: conventional agroforestry, MO: shade-less organic monoculture, MC: shade-less conventional monoculture

The availability of water underground is due to soil texture and competition with the roots of neighboring plants. The competition of the roots with neighboring plants was determined assuming that the reach of the roots is equivalent to a circumference equal to the projection of the treetop on the ground (Poorter 2017 com. pers.). The vegetation cover was measured in percentage above the crown of the tree (i.e., canopy cover) and next to the crown (i.e., neighbor cover).

Finally, we quantified the ambient temperature (°C) and absolute air humidity (g/m^3). These two measures were taken with a datalogger (PASCO Xplorer GLX Datalogger) by using a PASPORT Weather Anemometer Sensor, at 1.5 m height at four points around the tree simultaneously with transpiration. All measurements were averaged to have a value for each variable at the tree level.

Morphological leaf traits and transpiration

We collected the same leaves on which the transpiration was measured, to estimate the following leaf traits: The leaf area (LA, mm^2) describes the projected area of one side of the leaf (Pérez-Harguindeguy et al. 2016). The SLA (cm^2/g) describes the amount of leaf area per unit of biomass (Torrez et al. 2013). Both are involved in photosynthetic light capture. The LRWC (g/g) describes the relative amount of water that a leaf has per unit of biomass (Turner 1981) and assesses water status of a plant. The SD (stomata number/mm^2) refers to the number of stomata per unit of area. The SZ (μm) is defined as the size of occlusive cells. Former traits are involved in control hydric balance in the leaf (Lebrija-Trejos et al. 2010). The SD and SZ were measured by printing the leaf cuticle in transparent nail polish (Brewer 1992). The SZ was measured in 15 stomata per leaf. Microphotographs of leaf surface were taken with a 40 × trifocal optical microscope with a UCMOS 03100 KPA camera and analyzed with the OMAX ToupView 3.7 software (Daymond et al. 2011). The LA and SLA were measured using the method of Pérez-Harguindeguy et al. (2013), which calculates the SLA using microphotos processed with the software Image J to quantify the leaf area. The same leaf was then dehydrated in the leaf dryer and weighed at the Herbario Nacional de Bolivia (LPB). The SLA was calculated from the quotient of these two values. To calculate the LRWC, we weighted the fresh leaf mass in the field and the dehydrated leaf mass later in the herbarium. We divided the difference between fresh and dry leaf mass by the fresh leaf mass and multiplied by 100% (Turner 1981).

To estimate leaf transpiration rate, we measured the absolute air humidity with an infrared gas sensor connected to a datalogger (PASCO Xplorer GLX Datalogger) between 10:00 and 12:00 a.m., when the transpiration was the highest according to preliminary measurements. This was carried out by isolating a sunlit live leaf in a plastic container sealed with parafilm and recording the absolute humidity every second for two minutes. Measurements were not performed when raining or with wet leaves. Further, we divided absolute air humidity (cm^3) by LA values (cm^2) and multiplied by 3600 s. As result we obtained the amount of water vapor generated per unit of area (cm^2) and time (hour).

Statistical analysis

First we compared environmental variables and leaf traits between cocoa cultivation systems using linear mixed models (LMMs) with plots as a random effect. P-values were calculated from log-likelihood ratio tests (LRT), using LMMs with only the random effect as null models (Greven et al. 2008) with the significance level set at 0.05. Second, we evaluated the effect of environment and leaf traits on transpiration rate. We started with a full LMM including all the environmental variables and leaf traits as fixed predictor variables and the plots as a random variable and then created reduced models by simplifications of the full model. We performed a model comparison with the corrected Akaikes's Information Criterion (AICc) (Symonds and Moussalli 2011). We considered all the models, whose ΔAICc were less than 2, as best models (Burnham et al. 2011). We also calculated the importance value for each variable (i.e. the sum of Akaike weights' values of each variable over all models) to support variable selection. All statistical analyses were carried out with the statistical software R version 3.6.0 (R Core Team 2019) with MuMIn package for a model comparison (Barton 2019).

Results

Effects of cultivation systems on environmental variables and leaf traits

We found differences between cultivation systems for environmental variables and leaf traits. Canopy cover was higher in agroforestry than monoculture systems ($\chi^2 = 12.24$, $df = 1$, $P < 0.001$), but both temperature ($\chi^2 = 0.32$, $df = 1$, $P = 0.57$) and absolute air humidity ($\chi^2 = 1.25$, $df = 1$, $P = 0.26$) were similar between cultivation systems (Fig. 2a–c). No differences were found between the two cocoa varieties.

Among leaf traits, SLA was higher in agroforestry than monocultures ($\chi^2 = 12.56$, $df = 1$, $P < 0.001$) (Fig. 2d). In contrast, the SZ ($\chi^2 = 4.23$, $df = 1$, $P = 0.03$) and transpiration ($\chi^2 = 8.25$, $df = 1$, $P < 0.001$) were both higher in monoculture than agroforestry (Fig. 2e, f). The LRWC was slightly higher in agroforestry ($\chi^2 = 3.10$, $df = 1$, $P = 0.08$). Finally, we found no differences between cultivation systems for SD ($\chi^2 = 0.08$, $df = 1$, $P = 0.77$) (Fig. 2g, h).

Effects of environmental variables and leaf traits on transpiration

We found that transpiration was influenced by environmental variables and leaf traits. Temperature and canopy cover were the most important predictor variables, appearing in all of the six best models and had the highest importance values with temperature = 1.00 and canopy cover = 0.85. SLA followed these two predictors, appearing in five of the six best models and the third highest importance value = 0.65 (Table 1). The best model included temperature, canopy cover and SLA, which respectively explained 48, 16 and 11% of the variance in transpiration rate (Table 2). There is a positive relationship between temperature and transpiration (Fig. 3a) in both cultivation systems. By contrast, canopy cover and SLA had a negative relationship with the transpiration in the agroforestry systems (Fig. 3b, c).

Discussion

We found that cocoa plants developed larger stomata cells, smaller leaves and had greater transpiration in

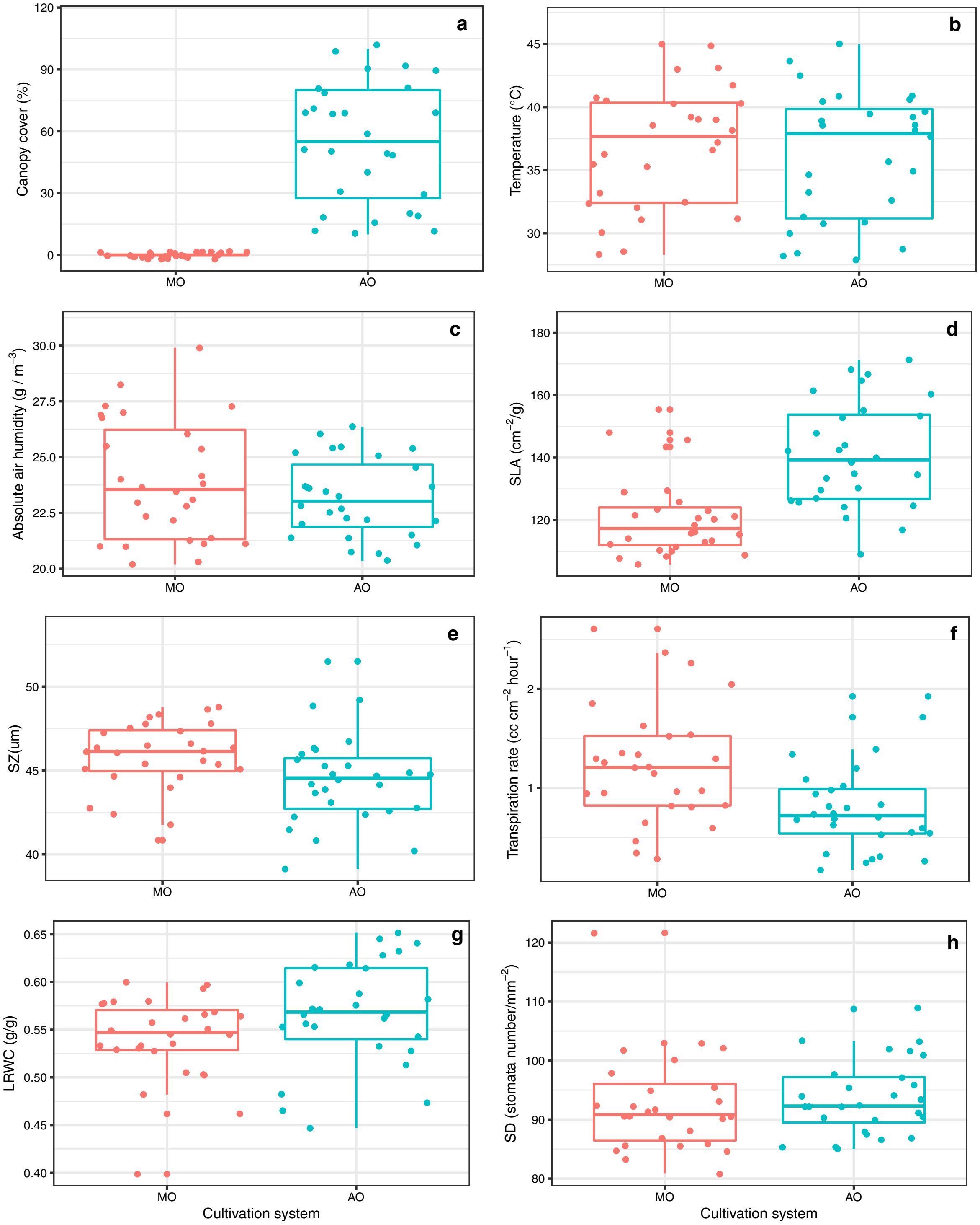

Fig. 2 Effects of cultivation systems, organic monoculture (MO) and organic agroforestry (AO) on: **a** canopy cover, **b** temperature, **c** absolute air humidity, **d** specific leaf area, (SLA), **e** stomata size (SZ), **f** transpiration rate, **g** leaf relative water content (LRWC) and **h** stomata density (SD). Horizontal lines in boxplots are medians, boxes indicate 25th and 75th percentiles, whiskers show data variability. Dots are recorded values (n = 56 trees). (Color figure online)

monocultures compared to agroforestry systems. These findings support our third hypothesis, which states no compromise between drought- and shade tolerance for *Theobroma cacao*. This may be due to cocoa plants under different cultivation systems having developed morphological and physiological adaptations associated with leaf traits. Also, it suggests that cocoa trees have great phenotypic plasticity. Thus, tolerance to shade is uncoupled from tolerance to drought or are coordinated with each other (Markesteijn et al. 2011) in response to microenvironmental conditions created in the cultivation systems.

Effects of cultivation systems on environmental variables and leaf traits

Cultivation systems have effects on microenvironmental conditions (Niether et al. 2018; Lin 2007) and on the expression of leaf traits (Martin and Isaac 2015). Our results showed that canopy cover was higher in agroforestry, whereas temperature and humidity were similar between cultivation systems. SZ and transpiration were higher in monocultures whereas SLA and LRWC were greater in agroforestry systems. In contrast to Hardy (1960) and Daymond et al. (2011) we found that the SD was similar between cultivation systems.

Long-term environmental variables such as canopy cover is greater in agroforestry (Somarriba et al. 2018). The establishment of companion trees in this cultivation system creates a vegetation cover that maintains constant shade conditions (Somarriba and Beer 2011). Shade may drive morphological (Liu et al. 2016) and physiological (Lin 2010) changes in plant species that grow under these conditions in order to achieve biological fitness. By contrast, we did not find differences in temperature and humidity between cultivation systems as has been previously reported (Niether et al. 2018). This is because these are short-term environmental variables susceptible to fluctuations in wind speed and cloudiness during a day (Unpublished data).

Monocultures are currently the most common cultivation system of cocoa worldwide. However, monocultures cause considerable more environmental damage than agroforestry would cause (Armengot et al. 2016). For instance, monocultures could have negative effects on the water physiology of plants (Tilman 1999). Our results show that cocoa plants in monocultures lose more water by increasing its transpiration rate than plants in agroforestry systems. This suggests that plants under shade conditions in agroforestry have a more efficient water use (WUE) by reducing the loss of water (Centritto et al. 2000; Köhler et al. 2014). Water loss by transpiration in

Table 2 Parameter estimation from the optimal linear mixed model (AICc = 49)

	Estimate	Standard error	t value
Intercept	− 0.9304	0.5109	− 1.82
Temperature	0.0744	0.0092	8.06
Canopy cover	− 0.00465	0.00145	− 3.20
SLA	− 0.00497	0.00278	− 1.79

Temperature, canopy cover and SLA were the most important predictor variables of changes in transpiration. Adjusted R^2 with only three most important predictors = 0.64

Table 1 Comparison of linear mixed models (LMM) constructed to test the effects of explanatory variables on transpiration

Model comparisons response variable ∼ predictor variables	AICc	*df*	ΔAIC	Weight
Transpiration ∼ **temperature (1.00)** + **canopy cover (0.85) + SLA (0.65)**	50.7	6	0.00	0.060
Transpiration ∼ **temperature (1.00)** + **canopy cover (0.85)**	51.3	5	0.59	0.044
Transpiration ∼ **temperature (1.00)** + **canopy cover (0.85) + SLA (0.65)** + neighbor cover (0.29)	52.2	7	1.53	0.028
Transpiration ∼ **temperature (1.00)** + **canopy cover (0.85) + SLA (0.65)** + SZ (0.32)	52.2	7	1.53	0.028
Transpiration ∼ **temperature(1.00)** + **canopy cover (0.85) + SLA (0.65)** + SZ (0.32) + LRWC (0.29)	52.4	7	1.71	0.025
Transpiration ∼ **temperature (1.00)** + **canopy cover (0.85) + SLA (0.65)** + LRWC (0.29)	52.4	8	1.75	0.025

The optimal model was that with the smallest AICc value. Importance values for each predictor variable are between parenthesis. Most important predictor variables are in bold font

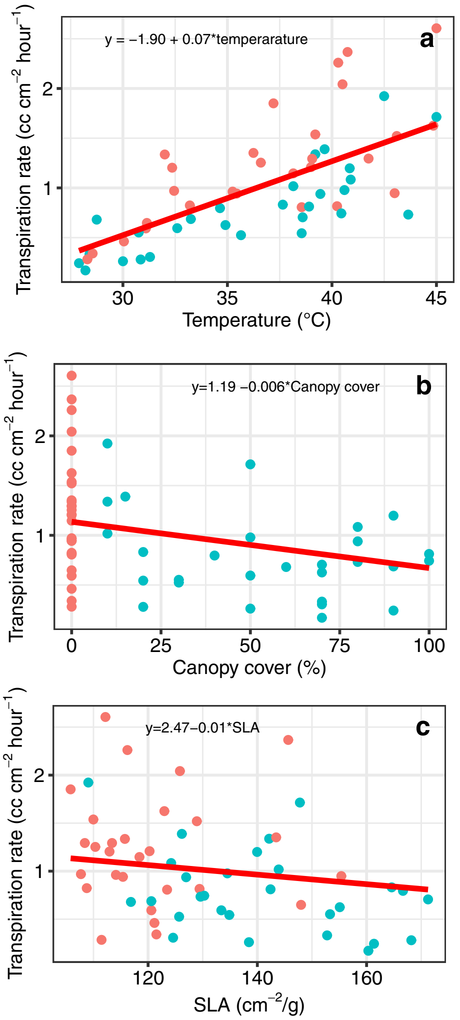

Fig. 3 Results of linear models (LM) show a positive relationship of transpiration with **a** temperature (adj. R-squared = 0.48, $F_{(1,54)} = 52.52$, $P < 0.05$), and negative relationship with, **b** canopy cover (adj. R-squared = 0.16, $F_{(1,54)} = 11.23$, $P < 0.05$) and **c** SLA (adj. R-squared = 0.11, $F_{(1,54)} = 7.97$, $P < 0.05$). Red dots correspond to monoculture and blue-light to agroforestry systems (n = 56 trees). Regression line was adjusted from fitted values using the coefficients from the optimal linear model performed for each predictor variable. (Color figure online)

monocultures may be associated with the larger SZ compared to agroforestry systems. Larger occlusive cells will have a large pore whereby a greater amount of water vapor can pass increasing stomatal conductance (Franks and Beerling 2009). A higher stomatal conductance was reported under full-sun conditions and it is positively correlated with transpiration in monocultures (Lin 2010; Puglielli et al. 2017).

Our results suggest that shade in agroforestry systems influences the foliar traits that contribute to reduce water use compared to monocultures. The response of plants to an increase in canopy cover may reduce the transpiration rate by increasing the SLA and LRWC. Plant species growing under shade conditions tend to display higher SLA values (Feng and van Kleunen 2014; Rozendaal et al. 2006). However, although LRWC was partially different between cultivation systems, more water was held in leaves collected from plants growing in agroforestry. To our surprise, SD was similar between cultivation systems suggesting that this trait has less plasticity than SLA and SZ.

Effects of environmental variables and leaf traits on transpiration

Environmental variables influence the transpiration of cocoa plants. Our results show that transpiration increases with the rise in temperature. However, it was significantly lower in agroforestry compared with monocultures. By contrast, an increase in canopy cover reduced the transpiration in the agroforestry systems.

For our study system, an increase in transpiration with temperature may be explained by the high leaf-to-air vapor pressure deficit (VPD) generated by the increase of temperature inside and around the leaf (Lin 2010; Naizaque et al. 2014). As the temperature increases, water is converted into water vapor in the chamber of mesophyll cells. This arises the evaporative demand into the atmosphere, leading to an increase in transpiration (Lin 2010; Niether et al. 2018). Similar to our findings, Naizaque et al. (2014) found an increase in transpiration with temperature in full-sun exposed plants of *Acca sellowiana* [O.Berg] Burret, in Colombia. This relationship was explained by the higher irradiance received by plants without canopy cover, such as in monocultures, leading to an increase in leaf temperature. For instance Kozlowski and Pallardy (1997) reported an increase in transpiration with an increase of irradiance. Additionally, direct irradiance and temperature cause a greater opening of stomata and consequently stomatal conductance (Hall 2001), contributing to water vapor loss. In the short term, daily fluctuations in temperature are responsible for generating major changes in transpiration.

By contrast, long-term environmental variables such as canopy cover contributed to reduced transpiration in agroforestry systems as was previously reported by Köhler et al. (2014) in Sulawesi, Indonesia and Centritto et al. (2000) in the Mediterranean region. Shade provided by companion trees in agroforestry reduce environmental stress caused by the direct incidence of solar radiation (Bote and Struik 2011; Niether et al. 2018) and may modify the expression of leaf traits of cocoa trees to reduce water loss by transpiration (Liu et al. 2016; Rozendaal et al. 2006).

Our findings suggest that temperature and canopy cover are the main environmental variables determining the transpiration of cocoa plants. In the long term, cultivation systems such as agroforestry systems may contribute to reduced water use by reducing the transpiration of cocoa plants (Centritto et al. 2000; Lin 2007). By contrast, monoculture systems are more susceptible to negative effects of increased temperatures on water loss via transpiration.

Leaf traits associated with leaf morphology had effects on transpiration rate. According to our findings, as SLA increases, the transpiration reduces significantly, but only in the agroforestry system. This negative relationship could be explained by changes in SLA (Bote and Struik 2011) and thickness (Puglielli et al. 2017), in response of shade conditions in agroforestry systems (Liu et al. 2016). We found that SLA was higher in the agroforestry systems compared to the monoculture. This pattern agrees with those reported by Centritto et al. (2000) for cherry saplings and Bote and Struik (2011) for coffee plantations. This supports the idea that SLA responds positively to shade conditions. By contrast, in monocultures SLA decreases as light intensity increases (Robakowski et al. 2003) to avoid water loss in drought stress conditions (Montanaro et al. 2009).

Other leaf traits of that may contribute to an explanation of this pattern are the SZ and LRWC. We found that SZ was smaller in agroforestry than monocultures systems, whereas LRWC was slightly higher in agroforestry than monoculture systems. Both traits were included in two of the six best models (Table 1). This suggests that transpiration in agroforestry could also be regulated by hydric traits associated with stomata operation (Köhler et al. 2014) and the capacity of leaves to store water in their tissue. Although we did not measure stomatal conductance, some studies have shown that stomatal conductance decreases as SLA increases (Puglielli et al. 2017). Thus, low transpiration under shade conditions in agroforestry may be explained by the interaction of high SLA, small occlusive cells and greater capacity to store water.

Conclusions

Cultivation systems have effects on microenvironmental conditions and leaf traits of cocoa trees. Canopy cover, temperature, and SLA were the main variables responsible for changes in the transpiration across cultivation systems. Cocoa plants have reduced the transpiration in agroforestry systems due to shade conditions created by canopy cover, which causes changes in the expression of morphological and physiological leaf traits such as SLA, SZ, and LRWC. Cultivation systems such as agroforestry may contribute to reduce water loss via transpiration in cocoa plants. Consequently, agroforestry systems could be used as an adaptative strategy to the negative effects of higher temperatures and less humidity in the context of climatic change.

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Author contributions The study was designed by Francisco Saavedra, Ernesto Jordan and Kasuya Naoki. Ernesto Jordan collected data in the field and laboratory. Francisco Saavedra and Kasuya Naoki conducted data analysis. Ernesto Jordan wrote the first draft of the manuscript. Early versions of the mansucript was wrote by Francisco Saavedra. Monika Schneider revised it critically early versions of the manuscript. All the authors discussed the results and commented on the manuscript.

Data availability The datasets generated and/or analyzed during the current study are available in the supplementary material or from the corresponding author on reasonable request.

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