



Combined effects of shade and drought on physiology, growth, and yield of mature cocoa trees

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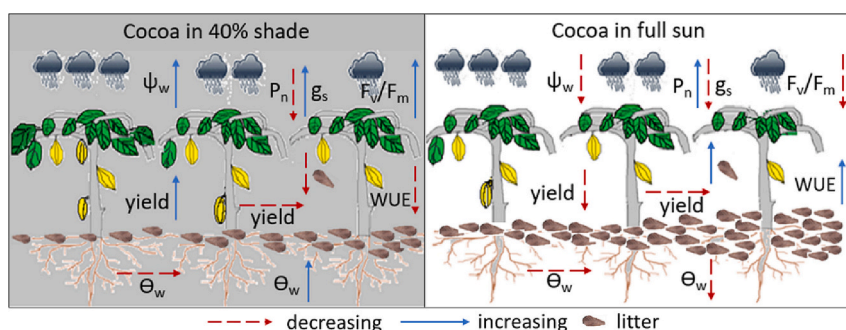
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HIGHLIGHTS

- Effects of shade and rainwater suppression were studied on mature cocoa trees.
- Rainwater suppression led to drought and decreased performances of the cocoa trees.
- Shade enhanced physiology, growth, and yield of the cocoa trees than the full sun.
- Shade will benefit cocoa but will not save it from negative effects of drought.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate models predict decreasing precipitation and increasing air temperature, causing concern for the future of cocoa in the major producing regions worldwide. It has been suggested that shade could alleviate stress by reducing radiation intensity and conserving soil moisture, but few on-farm cocoa studies are testing this hypothesis. Here, for 33 months, we subjected twelve-year cocoa plants in Ghana to three levels of rainwater suppression (full rainwater, 1/3 rainwater suppression and 2/3 rainwater suppression) under full sun or 40 % uniform shade in a split plot design, monitoring soil moisture, physiological parameters, growth, and yield. Volumetric soil moisture (Θ_w) contents in the treatments ranged between 0.20 and 0.45 m³m⁻³ and increased under shade. Rainwater suppression decreased leaf water potentials (Ψ_w), reaching -1.5 MPa in full sun conditions indicating severe drought. Stomatal conductance (g_s) was decreased under the full sun but was not affected by rainwater suppression, illustrating the limited control of water loss in cocoa plants. Although pre-dawn chlorophyll fluorescence (F_v/F_m) indicated photoinhibition, rates of photosynthesis (P_n) were highest in

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full sun. On the other hand, litter fall was highest in the full sun and under water stress, while diameter growth and carbon accumulation increased in the shade but was negatively affected by rainwater suppression. Abortion of fruits and damage to pods were high under shade, but dry bean yield was higher compared to under the full sun. The absence of interactions between shade treatments and rainwater suppression suggests that shade may improve the performance of cocoa, but not sufficiently to counteract the negative effects of water stress under field conditions.

1. Introduction

Cocoa (*Theobroma cacao* L.) is a shade-adapted plant that grows well in humid tropical conditions with regular rains and a short dry season (Pohlan and Perez, 2010), requiring a minimum of 1200 mm of water per year (Zuidema et al., 2005; Ameyaw et al., 2018). An extended dry period exceeding three months can reduce tree growth and yield of cocoa (Lahive et al., 2019) and cocoa thrives best when rain distribution is uniform along the year (Carr and Lockwood, 2011). About 70 % of the cocoa is produced in regions where most of the farms are rain-fed and characterized by 4–5 dry months (rainfall < 100 mm) (Wessel et al., 2015; Ruf et al., 2015; Lahive et al., 2019) indicating vulnerability of the cocoa tree to an already existing drought condition. This could explain why most of the cocoa farms are performing below the potential output, such as in West Africa where average yields are between 400 and 700 kg ha⁻¹ while potential yields in the sub-region are reported to be around 2000 kg ha⁻¹ and up to 6000 kg ha⁻¹ in other regions (Aneani and Ofori-Frimpong, 2013; Van Vliet and Giller, 2017; Bymolt et al., 2018; Asante et al., 2022). Climate projections foresee increases in frequency and severity of droughts (Läderach et al., 2013; Sylla et al., 2016; Ahmadi-pour et al., 2019), which causes concern for vulnerable cocoa farmers who have limited adaptive capacity to cope with the impact of weather events (Brian et al., 2022). Drought was causing an estimated 27 % yield loss in West Africa in the 1980s (Schroth et al., 2016) and is suggested to be the most serious threat to cocoa production (Carr and Lockwood, 2011). Responses to drought range from lower leaf transpiration rates and stomatal conductance of plants over decreased bean yields to death of trees (Rada et al., 2005; Schwendenmann et al., 2010; Abdulai et al., 2017; Gateau-Rey et al., 2018), confirming the negative effects of drought on cocoa production across the globe.

Drought occurs when soil water is reduced to the extent that plants can no longer extract sufficient water for normal life processes (Coder, 2018). High transpiration through the leaves but limited root water supply to the plants creates an imbalance (Anjum et al., 2011; Lamaoui et al., 2018) causing a reduced flow of water through the xylem to nearby cells and highly negative pressures in the xylem. Cell turgor declines, impairing the division and elongation of cells (Fahad et al., 2017), resulting in reduced growth and yield. Plants respond by stomatal closure, limiting water loss, CO₂ uptake and thus photosynthesis (Feller and Vaseva, 2014; Baligar et al., 2017). This reduces the production of assimilates and affects partitioning to reproductive organs. Thus, long drought periods impact flower production (Handley, 2016; Wuriandani et al., 2018) and cause flower abortion, reducing survival of pollinated flowers (Frimpong-Anin et al., 2014), pods sizes (Handley, 2016) and dry bean yield in cocoa (Abdulai et al., 2018; Gateau-Rey et al., 2018). The number of beans per pod, bean size, and weight are also reduced by drought (Handley, 2016).

Cocoa agroforestry is receiving increasing interest because yields are often higher under shade when fertilizer inputs are low (Ahenkora et al., 1974; Baligar et al., 2008; Asare et al., 2016). In addition, shade trees enhance functional biodiversity including sequestration of carbon, management of soil fertility and weeds, and biological control of pests and diseases (Wessel, 2001; Vaast and Somarriba, 2014; Vaast et al., 2016). As in other species, shade protects the photosynthetic machinery from photoinhibition (a condition that occurs under excessive light and degrades photosystem II), resulting in high photochemical efficiency and rate of electron transport of cocoa under shade, thus contributing to

increased production of assimilates and growth (Galyuon et al., 1996; Acheampong et al., 2013; Mensah et al., 2022). However, other studies point to negative effects of agroforestry under extreme drought (Moser et al., 2010; Abdulai et al., 2017; Gateau-Rey et al., 2018). Reductions in yield and mortality of cocoa trees may be due to belowground competition for both water and nutrients, aside from effects of reduced radiation. Competition for soil water and nutrients can be reduced by selecting the right species and/or management of shade (Asitoakor et al., 2022; Rigal et al., 2022), but it is unclear whether shade may play a positive role for cocoa trees subjected to drought stress. Recently, we showed that shade improved the physiological performance of cocoa seedlings but had limited effects on the response to high temperature stress (Mensah et al., 2022). Here, we investigate the hypothesis that shade reduces negative effects of drought on physiology, growth, and yield of cocoa trees. To test our hypothesis, we performed an ecosystem manipulation trial where mature cocoa trees were drought stressed by rainfall interception, while exposing them to full sun or uniform shade using artificial shade nets.

2. Experimental design

The experiment was conducted from April 2018 to March 2021 in a homogeneous, unshaded field at a cocoa farm in the Western North Region of Ghana (2°33'W, 6°23'N, 165 m a.s.l.). The site falls within the moist semi-deciduous forest zone. At the start of the experiment, the cocoa plants were 12 years old, 4.5 m tall and had an average diameter at breast height (DBH) of 8.5 cm. Spacing between the trees was on average 3 × 3 m.

A two-factor split plot design was used. The main plots consisted of two levels of shade (40 % shade and full sun) while the subplots consisted of three levels of rainwater suppression (full rainwater as control, 1/3 rainwater suppression, and 2/3 rainwater suppression). Selection of 40 % shade was based on earlier recommendations by Asare and David (2010) and Andres et al. (2018). The 1/3 and the 2/3 rainwater suppression levels were chosen to ensure that cocoa plants would be water stressed. As we did not control stemflow and possible lateral movements of water in the soil, we covered a larger proportion of the soil, expecting that this would result in a less-than-proportional reduction in soil water. The design was replicated in three blocks. Provision of shade was achieved using a shade net with a shading capacity of 40 % (Fig. 1A) raised above the cocoa canopy at 6.5 m from the ground.

Flat and slightly pending panels were raised in the rows of the cocoa trees at a height of 1 m, covered with plastic sheets (350 µm plane plastic sheets, Poly-products, Ghana) to lead the rainwater away from the plots (Fig. 1B).

In the 2/3 rainwater suppression treatment, the panels covered approximately 2/3 of the ground, while in the 1/3 rainwater treatments, about 1/3 of the ground surface was covered. Trenches were dug and lined with aluminium sheets leading the collected rainwater at least 10 m away from the treated plots. Each of the three blocks thus had six subplots (each subplot measuring 15 × 15 m and with 25 cocoa plants) including the six combinations of shade and rainwater suppression levels, resulting in a total of 18 subplots covering 0.5 ha. Main plots were surrounded by two border rows to eliminate border effects. Shade nets were installed the first time in July 2018 and water exclusion panels in October 2018. However, due to strong winds and mounting problems, blowing down shade nets and panels, they were fully effective from May 2019 and for the rest of the experiment (Fig. A.1).



Fig. 1. A) Shade nets providing 40 % shade suspended above the cocoa plants and B) Plastic sheets covering the spaces between cocoa plants to reduce precipitation. Photos: Eric Opoku Mensah, 2020.

All measurements were collected on the fourth matured leaf from the top phyllotaxy selected from three top or peripheral branches from the 9 middle plants in each subplot ($n = 9$ plants for 6 treatments and 3 replicates, totalling $N = 162$), using ladders to access the top of the crown.

2.1. Agronomic practices

The cocoa plants were pruned in April 2018 during the start of the experiment under a government-initiative program. Vertical shoots (chupons) were subsequently removed when observed. Weed control was achieved by slashing every two months during the rainy season (April – October) and every three months during the dry season (November – March). Control of mirids (*Sahlbergella singularis*, *Distantiella theobroma*, *Helopeltis* spp.) and other insects were done three times during the year; February, July, and September using Confidor (Imidaclopid; Kumark Company Limited, Ghana) and Akatsi master (Bifenthrin; Chemico Ltd., Ghana) at the recommended rates of 150 ml ha⁻¹ and 500 ml ha⁻¹, respectively (Baah et al., 2016). Black pod diseases (*Phytophthora megakarya*, *P. palmivora*) were controlled in July and in September every year using Ridomil Gold 66 WP (copper oxychloride and mefenoxam; Syngenta, Australia) at a rate of 50 g per 15 l of water. Regular removal of damaged and spotted pods was undertaken to reduce fungal sporulation. Parasitic mistletoes (*Tapinanthus bangwensis*) were removed with cutlasses regularly.

A composite soil sample was collected at the onset of the experiment and analysed. Soil textural analysis showed that the soil was a sandy clay

from 0 to 10 cm and clayey through the rest of the profile down to 160 cm (Determined at Soil Science Department, University of Ghana according to Beretta et al., 2014) while bulk density varied between 1.4 and 1.7 g cm⁻³ along the soil profile. Soil organic carbon, % total nitrogen and phosphorus were low (Table A.1). Application of Asaase wura cocoa fertilizer (an NPK fertilizer with 0-22-18 + 9CaO + 7S + 6MgO formulation) was done through broadcasting in May 2018 at a recommended rate of 400 kg ha⁻¹. Ammonium sulphate was placed in shallow basins around the trees at 70 g tree⁻¹ and about 40 cm from the base of the trees in May 2019 corresponding to 78 kg ha⁻¹. The NPK application was repeated in June 2020.

2.2. Environmental parameters

A weather station including a photosynthetic active radiation (PAR) sensor (S-LIA-M003), temperature/RH smart sensor (S-THB-M008), rain gauge sensor (S-RGx-M002) and HOBO data logger (H21-USB) (Onset Computer Corporation, USA) was installed in the nearby village, two kilometres away from the experimental site, and readings registered every 10 min. One hygrochron ibutton ((DS1923-F5 hygrochron, ibutton Link, United States) per block was hung below the cocoa canopy in the control plots 1.5 m above the soil surface, monitoring below canopy temperature and relative humidity every 10 min. Vapour pressure deficit (VPD) was calculated from temperature and relative humidity readings according to Howell and Donald (1995). The ibuttons were shielded under plastic bowls covered with aluminium foil.

Soil water content was monitored every second week from September 2019 to March 2021 with a Diviner soil moisture probe (Diviner 2000 Series II, Sentek Soil Moisture Sensors, Sentek Technologies, South Australia) using PVC pipes (NJPLAST GH uPVC 2"). Fifty-four pipes were installed in the field with equal distributions among the treatments. Volumetric moisture content was determined using the manufacturer's generic equations every 10 cm down to the 130 cm soil depth except sites with rocky pans, where measurements were monitored only to 90 cm soil depth.

2.3. Physiology

Leaf water potential was measured using a field Scholander pressure chamber (Pump-Up Chamber Instrument, PMS Instruments, USA) starting from November 2018 to December 2020. All measurements were taken at predawn (4,00–5:30 am) (Avila-Lovera et al., 2016), using small stems of about 1 mm thickness from the upper or peripheral branches from three selected plants in the middle row per subplot every month.

Diurnal trends of water potential were assessed in January, April, July, September, and December of 2019 and 2020 at five different time points (around 5:00 am, 9:00 am, 12:00 am, 3:00 pm and 6:00 pm). Measurements were done after leaves were sealed in aluminium foil for 30 min, over three-day periods with one block per day.

Non-destructive measurements of rate of net photosynthesis (P_n), transpiration (E), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i) and photosynthetic active radiation (PAR) were conducted with a CIRAS-3 portable gas analyser (PP systems, USA) in September 2019, December 2019, February 2020, April 2020, July 2020, and September 2020 between 10 am – 11 am. The fourth fully developed leaf of three selected branches from the top or the periphery of four selected middle plants per subplot were used for the measurements. Water use efficiency ($mmol\ mol^{-1}$) was calculated as the ratio of photosynthesis to transpiration. Measurements lasted for three days with one block per day, using natural light conditions with CO_2 set at $400 \pm 10\ \mu mol\ mol^{-1}$, cuvette temperature at $28 \pm 1\ ^\circ C$, 50 % of the ambient humidity and cuvette flow at $300\ cm^3\ min^{-1}$.

The ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) was measured using a mini-PAM photosynthesis yield analyser (Portable Chlorophyll Fluorometer - Heinz Walz GmbH, Germany) in darkness from 4:30–5:30 am in the same months as gas exchange was measured. Minimum fluorescence in dark-adapted state (F_0) was read with a measuring beam at low light intensity ($<0.02\ \mu mol\ m^{-2}\ s^{-1}$) while maximum fluorescence in dark-adapted state (F_m) was obtained after a saturating pulse was applied (about $5500\ \mu mol\ m^{-2}\ s^{-1}$ PAR for a duration of 0.7 s) (Chen et al., 2012). Variable fluorescence (F_v) was calculated as the difference between F_m and F_0 ($F_v = F_m - F_0$) while maximum photochemical efficiency of the photosystem II was determined as $F_v/F_m = (F_m - F_0)/F_m$.

2.4. Litter fall, stem growth and carbon accumulation

Plant litter fall was collected from October 2018 to December 2020. Four wooden boxes of $0.25\ m^2$ base area and 0.5 m height (Ofori-Frimpong et al., 2007; Brando et al., 2008; Triadiati et al., 2011) were placed on each subplot between the middle nine plants (Dawoe et al., 2010; Paudel et al., 2015). Each litter box was placed on bamboo sticks 5 cm above the soil surface to prevent decay. Litter from the four boxes per subplot were pooled monthly, oven dried at $70\ ^\circ C$ for 48 h (Schwen-denmann et al., 2010) and weighed to measure biomass leaf yield per treatment.

Dendrometer bands (DBM80 manual band dendrometer, ICT International) were placed around the stems of two plants from each subplot to measure stem expansion at 90 cm from the base of the stem. Growth of stem was observed using the sliding spring scale on the bands, measuring between 10 and 11 am monthly from November 2018 to December 2020.

Effects of shade and rainwater suppression on carbon accumulation were determined from the aboveground biomass (AGB) of the cocoa plants. AGB was calculated using the allometric equation for tropical trees proposed by Chave et al. (2014) where $AGB_{tree} = 0.0673 \times (\rho D_{150}^H)^{0.976}$, ρ = tree density ($0.42\ g\ cm^{-3}$ for cocoa as indicated by Chave et al., 2006 and Wade et al., 2010); D_{150} = initial diameter (at 150 cm-height) of the cocoa trees collected before the start of the treatments plus the stem expansion (cm) taken from the dendrometer band readings at the last month of rainwater suppression; The H in the equation is the average cocoa tree height (m) at the start of the experiment. We assumed that the stem expansion where the dendrometer bands were placed was equivalent to the stem expansion at the D_{150} , and that tree height did not increase during the experiment. The latter may have led to an underestimation of C accumulations in the cocoa plants after the treatments. AGB of the cocoa trees was converted into $Mg\ ha^{-1}$ by using the average spacing between trees (Macias et al., 2017; Afele et al., 2021). Carbon accumulation was calculated as $AGB \times F_c$; where $F_c = 0.5$ (F_c = carbon fraction) (IPCC, 2003; Somarriba et al., 2013).

2.5. Yield

Flower production and canopy density were assessed on a visual scale from 0 to 5; a score of zero indicated no flowers or no leaves and a score of 5 indicated maximum flowering intensity or a dense canopy. These assessments were conducted on the middle nine trees of each subplot. The total number of individual flowers produced per tree was quantified by estimating the number of flower cushions and the number of flowers per cushion at the section of the stem from 50 to 150 cm from the base of the tree. The estimates were based on images (Fig. A.2) of stems of ten plants, selected because of their differences in flowering intensity score during the peak flowering month (July 2018). On these trees, the number of flower cushions was counted and averaged for each level of score. The number of individual flowers of a flower cushion of one hundred randomly selected cushions was counted and averaged (Average number of individual flowers per cushion = 16). The total number of flowers per plant along the selected part of the stem was then calculated as the product of the number of flower cushions per score per plant and the average number of individual flowers per cushion. Flower production was measured monthly on the middle nine plants of each subplot from September 2018 to March 2021.

Young pods ($\leq 6\ cm$ length or $\leq 3\ cm$ width) were considered as cherelles. The numbers of healthy and dead cherelles on the stem from 50 to 150 cm from the base were counted monthly to assess the transition of flowers to cherelles and pod formation. Damaged pods and aborted cherelles were removed after each monthly count of the cherelles. Finally, the total number of pods on the whole tree was counted, followed by harvesting and recounting of healthy ripe pods to determine pod yield per tree.

Weight, length, and width of pods were measured on ten randomly selected pods per subplot after each harvest during the peak harvesting months (September, October, and November from September 2018 to March 2021). The number of beans per pod excluding the pulps was counted and weighed. After weighing, beans were pooled per subplot and the weight of 100 randomly selected beans was recorded. Beans were treated separately for each subplot and were covered with plantain leaves, labelled, and kept under a heap of cocoa beans for fermentation for six days. Beans were then sun-dried for seven days and weighed to compute dry bean yield per hectare and season (two seasons annually, with the major season being from September the previous year to March the following year, while the minor season was from April to August in the same year), following a modified model of Lachenaud (1984) and Wibaux et al. (2017):

$$Y = \frac{m \times NPH \times NBP \times MDB(g)}{n \times 1000g \times kg^{-1}}$$

where;

Y = Yield $^{-1}\text{ha}^{-1}\text{season}^{-1}$ ($\text{kg ha}^{-1}\text{ season}^{-1}$)

m = Total number of trees per hectare (1110 trees per hectare with 3 m $\times$ 3 m planting distance)

n = Number of trees used for yield measurements ($n = 9$)

NPH = Average number of healthy pods harvested per season

NBP = Average number of beans per pod

MDB = Average mass per dry bean

1000 g kg^{-1} = Factor to convert yield in g to yield in kg

2.6. Data analyses

The statistical analyses fall into 3 groups depending on the sampling schemes. The first group investigated the influence of shade (2 levels: full sun, 40 % shade) and rainwater suppression (3 levels, full rainwater, 1/3 rainwater suppression, 2/3 rainwater suppression) on pods and beans physical characteristics. The second group investigated the influence of shade, rainwater suppression and day with either 6 levels (Sep-19, Dec-19, Feb-20, Apr-20, Jul-20, Sep-20) on gas exchange and chlorophyll fluorescence; or 26 levels (Nov-2018, Dec-2018 up to Dec-2020) on water potential, stem expansion and litter fall. The third group investigated the influence of shade, rainwater suppression, and soil depth (13 levels, 0–10 cm, up to 120–130 cm) on soil moisture. The effects stated above were included as fixed effects together with their interactions in a linear normal model. In addition to the fixed effects, the statistical models also included random effects of plant id as well as temporal correlated residuals (for the second group) and spatial-temporal correlated residuals (for the third group) to model potential correlations between the observations. Thus, this resulted in linear mixed effects models, which were estimated using the nlme-package (Lindstrom and Bates, 1990) in the R statistical software (version 4.1.2, R Core Team, 2021).

Conditions of homoscedasticity were verified by plotting residuals against predicted values, and the normality of residuals were validated by normal quantile plots. When necessary, response variables were transformed to verify these conditions. Particularly, data from diurnal measurements of water potential, transpiration, sub-stomatal CO_2 concentration, stomatal conductance, water use efficiency, stem expansion and litter fall were log-transformed while soil moisture values were square-root transformed. Moreover, for models with correlated residuals, the correlation structure was selected from a pool of potential correlation structures via the Akaike Information Criteria (AIC).

After the initial model selection and validation, the models were reduced via the backward selection method (Pope and Webster, 1972), and the final models were used for reporting results. Predicted means were separated with multiple comparisons using Tukey's Honestly Significant Differences (Tukey HSD).

3. Results

3.1. Environmental conditions, plant water potential and soil moisture

Rainfall varied during the experiment, being more evenly distributed during 2019 than in 2020. The annual rainfall was 1110 mm in 2019 and 1250 mm in 2020. Still, both years had dry conditions around February and August, however, more pronounced in 2020. Photosynthetic active radiation (PAR) values at the experimental site also varied based on the day of measurement (Fig. A.3). Although the shade nets were supposed to provide 40 % shade, measurements with the portable gas analyser suggested that reductions in PAR values were variable and on average slightly higher as values ranged between 34 and 58 % of the radiation under full sun, depending on the month (Fig. A.4). Relative humidity under the nets was higher than in the full sun, corresponding to an average increase of 4–11 % depending on the month. The corresponding sub-canopy temperatures were on average 1.5–3.1 °C lower in shade

compared to full sun plots while VPD were 0.04–0.28 kPa higher in the full sun (Fig. A.5). Neither relative humidity nor temperatures were affected by the rainwater suppression treatments (Table A.2).

Plant water potential (ψ_w) at predawn was significantly affected by shade, rainwater suppression and day of measurements. Under shade, ψ_w values ranged between -0.7 and -0.15 and were between 0.14 and 0.05 MPa higher than the values obtained in the full sun plots during the wet months and between 0.28 and 0.06 MPa higher during the dry months (Table A.2, Fig. 2). Among the rainwater suppression treatments, leaf water potential was very low in the 2/3 rainwater suppression plots compared with the 0/3 and the 1/3 plots. For example, 2/3 rainwater suppression plots in the full sun showed ψ_w values as low as -0.9 MPa compared with the full rainwater plots with -0.6 MPa in the same month.

Water potentials were more negative in the dry months between November to March compared to the wet months and varied in parallel with soil moisture contents (Θ_w). The values of Θ_w ranged between 0.20 and 0.45 m^3m^{-3} among the treatments (Fig. 2). While overall Θ_w was higher under the shade compared to the sun, this depended on the soil depth, as differences were larger in deep soil layers (Fig. A.6). However, 2/3 rainwater suppression plots in the full sun were moister for depths from 0 to 50 cm compared with the shade for reasons that we cannot account for. Generally, ψ_w was high during predawn measurements and low at midday (Fig. A.7). Midday ψ_w of the 2/3 rainwater suppression plots were -0.7 MPa during the rainy season but reached values as low as -1.5 MPa in the dry season.

3.2. Photosynthesis

Photosynthesis (P_n) was high in the wet months (July–September) and tended to be highest in the full sun treatment. However, the effect of shade depended significantly on the month of measurements with differences between the shade and the sun being small in the dry months (Fig. 3, Table A.2).

Effects of rainwater suppression also depended significantly on the month of measurement, with the lowest values observed in the rainwater-suppressed treatments and differences increasing over time. Sub-stomatal CO_2 concentrations (C_i) were high under shade especially in February, April, and July 2020 and increased from full rainwater to 2/3 rainwater suppression plots in both shade and full sun.

Transpiration (E) was significantly higher under shade than in the full sun, but differences between rainwater suppression levels were not significant (Fig. 3, Table A.2). Values were between 1.5 and 5.5 $\text{mmol m}^{-2}\text{ s}^{-1}$ and increased under shade and in the wet months. This was caused by stomatal conductance (g_s) which was higher under shade than in the full sun. Again, the effects of rainwater suppression levels were not significant. Water use efficiency (WUE) showed significant interactions between shade levels, rainwater suppression and months, and was higher in the full sun than in shade in the full rainwater and 1/3 rainwater suppression treatments in the relatively wet months of April and July 2020.

Dark adapted chlorophyll fluorescence was affected by interactions between shade and rainwater suppression, and by the time of measurement (Table A.2). Chlorophyll fluorescence was low during the dry months of December and February 2020 (Fig. 3) in all the treatments and was very low in the full sun treatments compared with the shade treatments. It decreased with increasing level of rainwater suppression, indicating photoinhibition in these treatments.

3.3. Litter fall, stem expansion and carbon accumulation

Effects of shade on litter production depended on the level of rainwater suppression and on the time of collection (Table A.3). In the dry season (November – February), litter production peaked and increased in the full sun plots compared to the shade plots (Fig. 4).

Rainwater suppression increased litter fall, with the 2/3 rainwater

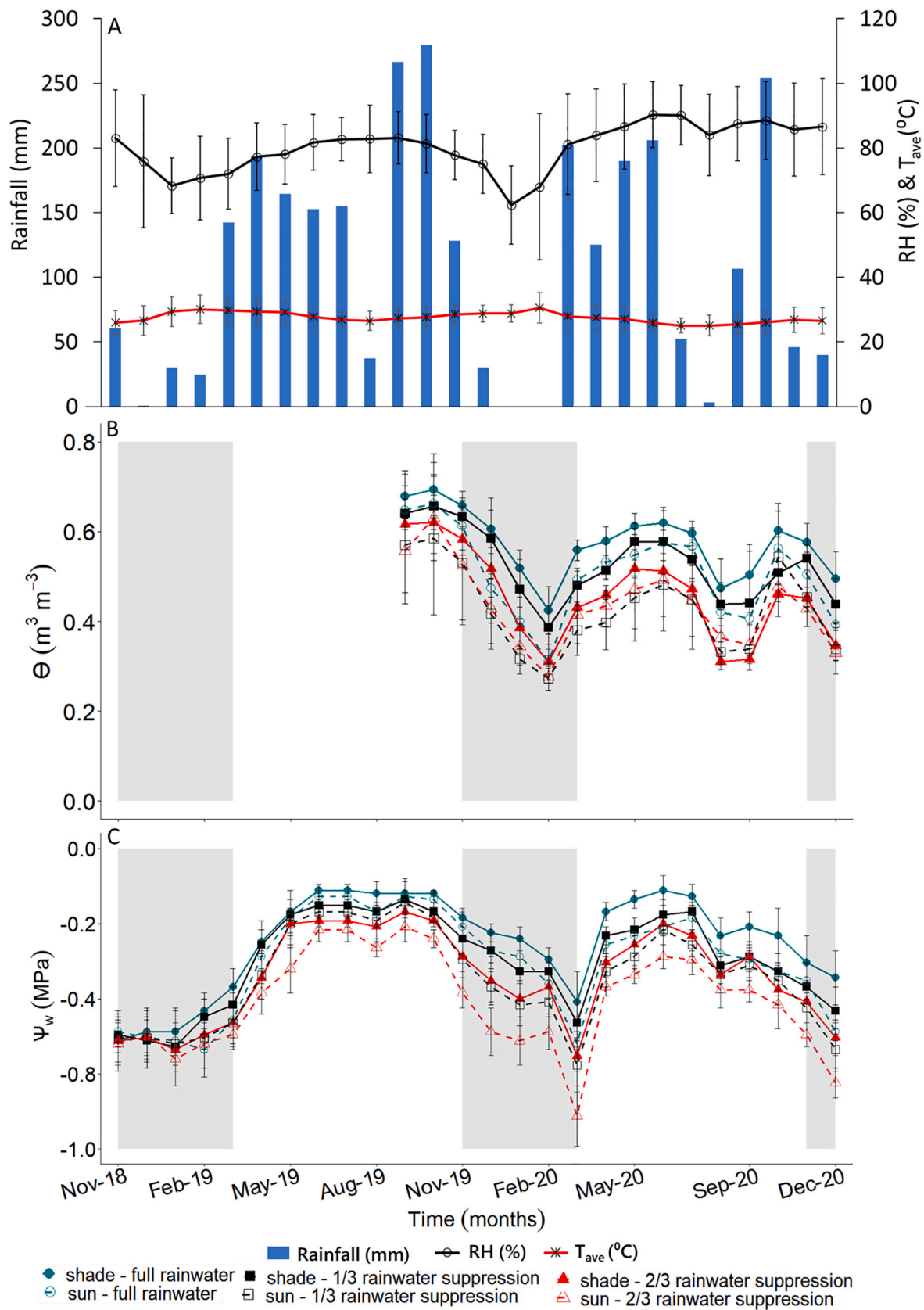


Fig. 2. Monthly variations of rainfall (blue bars), temperature (red line) and relative humidity (black line) measured from Nov. 18 - Dec. 2020 (A), volumetric soil moisture content (Θ_w) measured from Aug. 2019 - Dec. 2020 (B) and predawn water potential measured from Nov. 2018 - Dec. 2020 (C) as affected by shade and water suppression levels. Soil moisture could not be measured earlier due to difficulties encountered with the moisture probe. Bars indicate standard error ($n = 3$). Dark background represents dry periods.

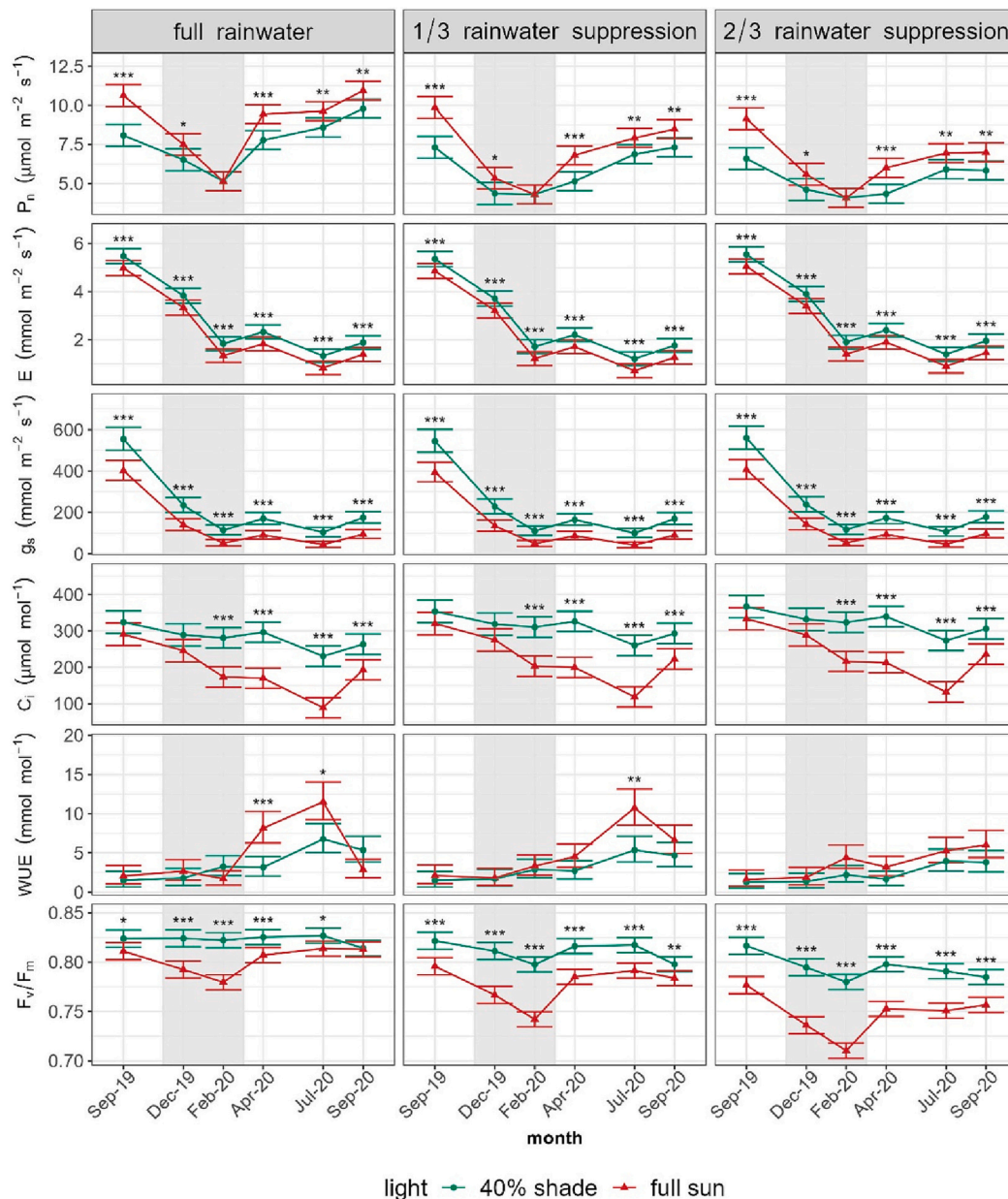


Fig. 3. Effects of shade and water suppression on cocoa photosynthesis (P_n), transpiration (E), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), water use efficiency (WUE) and chlorophyll fluorescence (F_v/F_m). Bars indicate standard error ($n = 3$), and stars indicate significant differences between shade and full sun (***) $P < 0.001$, (***) $P < 0.01$, (*) $P < 0.05$). Dark background represents dry periods.

suppression treatments in the full sun conditions giving the highest monthly litterfall of 1.2 Mg ha^{-1} in February 2020 (Fig. 4A). Annual litter fall in shade ranged between 3.6 and 6.2 Mg ha^{-1} for the rainwater suppression levels while under the full sun conditions, litterfall was 4.4 Mg ha^{-1} for full rainwater plots and 6.7 Mg ha^{-1} in 2/3 rainwater suppression plots.

High litter fall coincided with low canopy density, which showed interactions between shade, rainwater suppression and month of assessment. Values were generally high in full rainwater plots in shade in the wet months but were low in the 2/3 rainwater suppression treatments and under the full sun (Fig. 4B).

Stem expansion was affected by rainwater suppression and month of measurement but not by shade (Table A.3), increasing at the start of the wet season (between March to July) but declining towards the end of the year for all treatments (Fig. 4C). In the dry months of January and February 2020, growth almost stopped. Yearly total stem expansions

were between 3.9 and 6.1 mm year^{-1} while monthly changes varied between -1.0 to $2.5 \text{ mm month}^{-1}$. Expansion rates of plants in full rainwater plots were on average 2.7 mm year^{-1} higher than values in 1/3 rainwater suppression plots and 3.3 mm year^{-1} higher than values in 2/3 rainwater suppression plots. On the other hand, values for the 1/3 rainwater suppression plots and the 2/3 rainwater suppression plots did not differ significantly.

Carbon accumulation was not affected significantly by the shade levels, whereas the effects of rainwater suppression were only significant at 10 % (Table A.3). On average, $4.54 \text{ Mg C ha}^{-1}$ was accumulated by the 12-year-old cocoa plants before the start of the experiment. After almost three years of shade and rainwater suppression, carbon accumulation increased by about $2.73 \text{ Mg C ha}^{-1}$ under shade and $2.33 \text{ Mg C ha}^{-1}$ under full sun conditions. Carbon accumulation was on average $1.04 \text{ Mg C ha}^{-1}$ lower in the shade - 2/3 rainwater-suppressed plots than in the full rainwater treatments, while under the full sun conditions, it

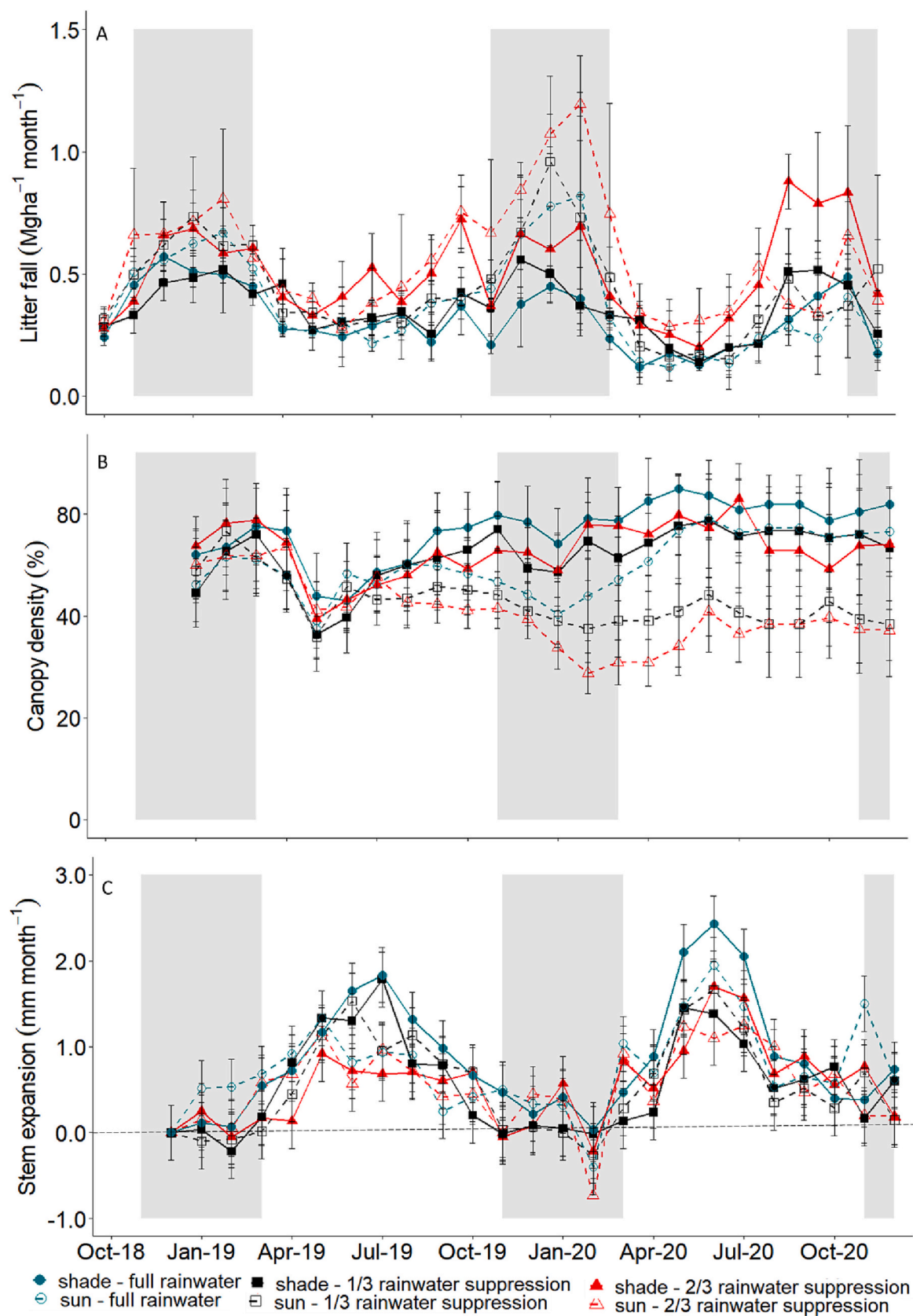


Fig. 4. Effect of different levels of shade and water suppression on litter fall (A), canopy density (B) and stem expansion assessed as diameter increase of cocoa (C) monitored for two years and three months. Data on litter fall were measured from Oct. 2018 – Dec. 2020; stem expansion from Dec. 2018 – Dec. 2020; and canopy density from Jan. 2019 – Dec. 2020. Bars indicate standard error ($n = 3$). Dark background represents dry periods.

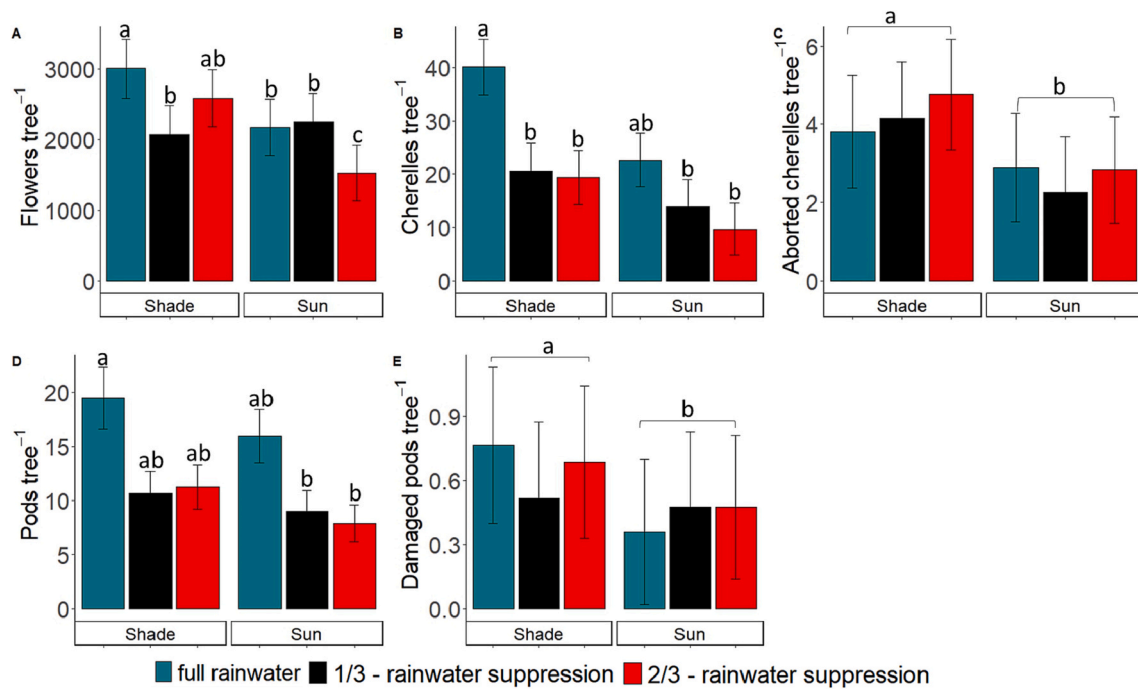


Fig. 5. Effects of shade and water suppression on annual average number of flowers (A), cherelles (B), aborted cherelles (C), pods (D) and damaged pods (E) at 1.5 m of the cocoa trees. Means with different letters are statistically significant at $P < 0.05$ (Tukey's HSD). Number of pods, flowers and cherelles were assessed from 0.50 m to 1.5 m of the stem of the cocoa tree monthly from January 2019 to December 2020. Bars indicate standard error ($n = 3$).

Table 1

Effects of shade and water suppression on pods and beans physical characteristics. Means are $\pm$ standard error ($n = 3$). Means in a row not sharing letters are significantly different at $p < 0.05$ according to Tukey's HSD.

Shade levels	Shade			Sun		
Rainwater levels	Full rainwater	1/3 rainwater suppression	2/3 rainwater suppression	Full rainwater	1/3 rainwater suppression	2/3 rainwater suppression
Pod physical appearance						
Pod weight (g pod ⁻¹)	516 $\pm$ 122a	475 $\pm$ 151abc	486 $\pm$ 158ab	479 $\pm$ 104ab	455 $\pm$ 115bc	431 $\pm$ 117c
Pod length (cm-pod ⁻¹)	15.6 $\pm$ 1.6a	15.0 $\pm$ 1.2ab	15.2 $\pm$ 0.5ab	15.2 $\pm$ 1.7ab	15.1 $\pm$ 0.2ab	14.5 $\pm$ 0.4b
Pod diameter (cm pod ⁻¹)	8.5 $\pm$ 0.3a	8.2 $\pm$ 0.1b	8.2 $\pm$ 0.3b	8.1 $\pm$ 0.4b	8.0 $\pm$ 0.9b	8.1 $\pm$ 0.2b
Length/Diameter	1.8 $\pm$ 0.2b	1.8 $\pm$ 0.2b	1.8 $\pm$ 0.2b	1.9 $\pm$ 0.1a	1.9 $\pm$ 0.2a	1.8 $\pm$ 0.2b
Bean quantity and weight						
Beans/Pod	36.5 $\pm$ 1.2a	35.9 $\pm$ 2.2a	36.7 $\pm$ 3.7a	34.4 $\pm$ 5.6b	34.3 $\pm$ 2.6b	34.8 $\pm$ 3.6b
Total bean weight (g pod ⁻¹)	123.5 $\pm$ 14.7a	113.8 $\pm$ 12.1abc	117.4 $\pm$ 9.1ab	114.9 $\pm$ 44.0abc	103.2 $\pm$ 15.2c	107.0 $\pm$ 18.2bc
% Beans weight/Pod	24.5 $\pm$ 4.1ab	24.6 $\pm$ 4.8ab	24.4 $\pm$ 3.8ab	23.7 $\pm$ 3.9ab	22.9 $\pm$ 3.6b	25.1 $\pm$ 4.2a
Fresh weight (g bean ⁻¹)	3.5 $\pm$ 0.4a	3.2 $\pm$ 0.1ab	2.8 $\pm$ 0.2c	3.0 $\pm$ 0.2bc	2.7 $\pm$ 0.5 cd	2.4 $\pm$ 0.2d
Dry weight (g bean ⁻¹)	1.3 $\pm$ 0.7a	1.2 $\pm$ 0.1ab	1.2 $\pm$ 0.1ab	1.3 $\pm$ 0.1ab	1.2 $\pm$ 0.1ab	1.1 $\pm$ 0.1b
Moisture (g bean ⁻¹)	2.2 $\pm$ 0.4a	1.9 $\pm$ 0.1ab	1.6 $\pm$ 0.1c	1.7 $\pm$ 0.1bc	1.5 $\pm$ 0.4 cd	1.3 $\pm$ 0.2d

was about 0.61 Mg C ha⁻¹ lower compared with the full rainwater plots (Table A.5).

3.4. Yield

The number of flowers was influenced by interactions between shade and rainwater suppression and was highest in the shaded full rainwater plots, reaching 3000 tree⁻¹ year⁻¹ for the one-meter section of the stem that was evaluated. Values were lowest in the full sun plots under 2/3 rainwater suppression, where averages were ca. 1600 tree⁻¹ year⁻¹ (Table A.3, Fig. 5A).

Though the number of flowers was high, the number of cherelles was much low with values between 10 and 40 tree⁻¹ year⁻¹. Thus, only about 2 % of the flowers developed into cherelles and only about 1 % developed into mature pods (Fig. 5B & D). The shaded plants showed higher numbers of cherelles compared with the full sun plants (Fig. 5B).

On the other hand, trees under rainwater suppression in both the shade levels had low numbers of cherelles. Mean pod numbers varied between 7 and 20 tree⁻¹ year⁻¹ with plants under the shade producing more pods than plants under the full sun and lower numbers in the water-suppressed treatments (Fig. 5D). There were large differences among individual trees with pod counts ranging from 0 to 79 pods tree⁻¹ year⁻¹. The numbers of aborted cherelles and damaged pods were not significantly affected by the rainwater suppression levels but were significantly higher under the shade nets than under the full sun (Fig. 5C & E).

Average pod weight ranged between 431 g and 516 g among treatments (Table 1), with pods from the shade being 20 to 55 g heavier than pods from the full sun conditions.

Rainwater suppression reduced pod weight by about 10 % in the sun and 8 % in the shade. Also, pod length and diameter were negatively affected (Table 1). The average number of beans per pod varied between

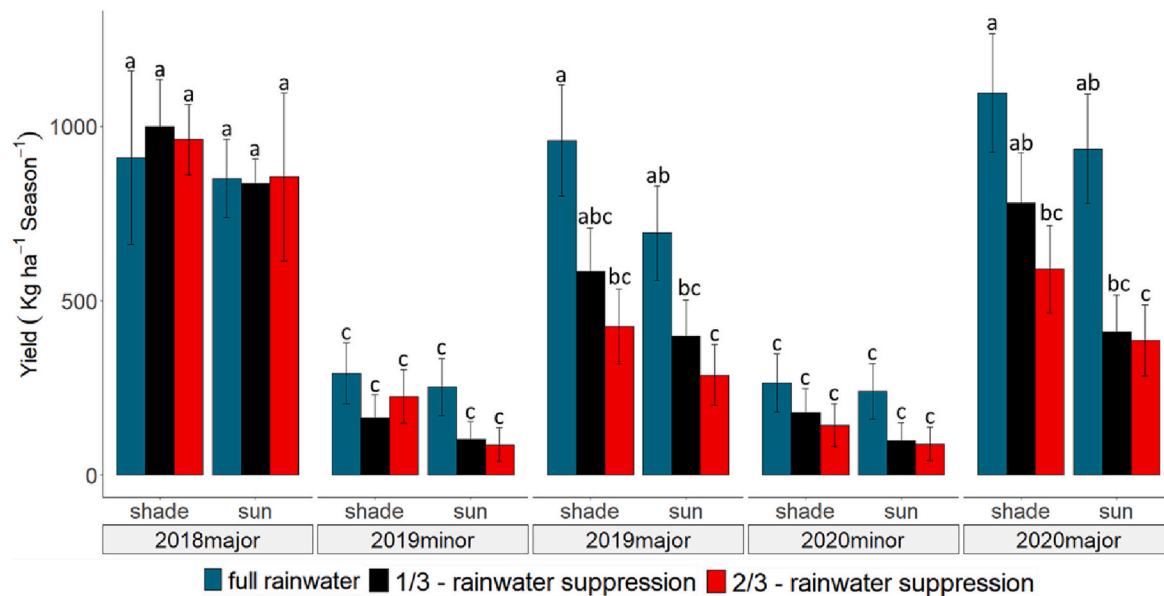


Fig. 6. Effect of shade and water suppression on yield of cocoa plants between September 2018 and March 2021. Means with different letters are statistically significant at $P < 0.05$ (Tukey's HSD). Bars indicate standard error ($n = 3$).

34 and 37 with plants in the shade producing 2 to 4 beans more per pod than plants in the sun. Hence, the total bean fresh weight per pod was 103 g to 124 g for the shade trees receiving full rainwater, yielding almost 10 g more per pod than plants in the sun. Total bean fresh weight per pod was reduced by about 8 % in the shade and 10 % in the full sun as rainwater suppression increased from full rainwater to 2/3-rainwater suppression. Similar tendencies were seen for fresh and dry weight per bean.

Dry bean yield (in $\text{kg ha}^{-1} \text{ season}^{-1}$) was significantly higher under the shade than in the sun, being 12 to 45 % higher at all levels of rainwater suppression (Table A. 4, Fig. 6). Rainwater suppression decreased yields to <50 % in the 2/3 rainwater suppression treatment, compared to the full rainwater treatment. Yield was generally low in the minor seasons with averages between 87 and 293 $\text{kg ha}^{-1} \text{ season}^{-1}$ but was much higher in the major season ranging from 286 to 1105 $\text{kg ha}^{-1} \text{ season}^{-1}$. Differences were especially pronounced during the 2020 major season. Analysing the seasons individually, differences between treatments were non-significant for the 2018 major season (while the treatments were not yet fully installed) and the minor seasons in 2019 and in 2020, but significant for the major seasons in 2019 and in 2020.

4. Discussion

To our best knowledge, this is the first experiment to investigate the combined effects of shade and limitations in water supply under field conditions in mature cocoa trees. Our setup with shade nets and plastic panels resulted in clear differences in light levels, soil water contents and water status of the trees, and cocoa plants responded by changes in physiology, growth, and yield. However, as we will discuss below, the absence of interactions between shade and rainwater suppression treatments for important characters did not support our hypothesis that shade would fully compensate for the negative effects of drought.

Even though levels of drought stress were not lethal, trees were negatively affected by drought, having decreased growth rates, larger leaf fall, thin foliage, and a possible decrease in biomass accumulation. The low leaf water potentials in rainwater-suppressed plants reduced photosynthesis and led to photoinhibition as indicated by the low values of F_v/F_m , commonly seen in drought-stressed plants (Janusz et al., 2006; Bae et al., 2008; Moser et al., 2010; De Almeida et al., 2016).

The absence of effects of rainwater suppression on stomatal

conductance suggests a poor stomatal regulation in response to drought in cocoa. Stomatal conductance (g_s) was significantly affected by shade levels but not by rainwater suppression levels, showing limited control of water loss in cocoa (De Almeida and Valle, 2007). The relationship between stomatal conductance and leaf water potential differs among groups of plants (Qaderi et al., 2019). It should be noted, however, that stomatal conductance was generally low at the four last assessments, to some degree coinciding with periods of low precipitation. Carbon dioxide (CO_2) concentrations inside the leaf increased with increasing rainwater suppression despite the lack of differences in stomatal conductance, suggesting non-stomatal limitations to photosynthesis (Brodrick, 1996). At high-stress levels, impaired photosynthetic metabolism may be caused by weakening of photosystem II and alteration of the thylakoid membrane proteins (Marino et al., 2018).

Shade improved microclimatic conditions reducing average air temperatures and maximum temperatures compared to the full sun plots, while relative humidity was higher. Plants under full sun were exposed to high solar radiation combined with high temperatures. In the dry months of March and April, below canopy temperatures were as high as 42 °C, values above the reported optimum for growth of 24 °C - 34 °C (Gomes and Kozłowski, 1987; Najihah et al., 2018) and for photosynthesis of 31 to 35 °C (Balasimha et al., 1991; Yapp, 1992; Mensah et al., 2022). Though reported cases show cocoa plants surviving at 40 °C (Valle et al., 1990), physiological activities of key elements of photosynthesis, including PSII activation, ATPase activity and the carbon assimilation process, are impaired (Mathur et al., 2010; Chen et al., 2012; Carrion-Tacuri et al., 2013). Hence shade, through the reduction of temperatures, may reduce the risks of high temperatures. The evidence of large differences in soil moisture content between shade and full sun in deep soil layers might be due to high relative humidity under shade resulting in reduced evapotranspiration and facilitating water retention at the deep soil layers. This would be an advantage under shade, conserving soil moisture for use during dry periods.

At the same time, solar radiation for full sun conditions was high with PAR ranging between 1200 and 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, much above the light saturation point of 300 to 550 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for cocoa (Avila-Lovera et al., 2016; Salazar et al., 2018; Mensah et al., 2022). This is another likely cause for the photoinhibition that was indicated by the low F_v/F_m values. Values averaging between 0.65 and 0.77 have been reported in full sun cocoa plantations (Galyuon et al., 1996; Bae et al.,

2008) but values of 0.8 noted under shade showed intact PSII systems (Acheampong et al., 2013; De Araujo et al., 2017). Still, the plants in full sun conditions were able to maintain higher photosynthetic rates per unit area compared to trees under shade, indicating that photosynthesis was limited by light under shade. This seems to have been compensated for by the lower leaf fall and high canopy density under shade. A higher leaf area with high PSII activity may have contributed to higher gross canopy photosynthesis under shade compared to full sun, in turn leading to higher rates of pods formation and better development of pods under shade. Unlike plants under shade, photoinhibition experienced by the plants under full sun may have shortened leaves' life span and increased litter fall, resulting in lower or reduced gross canopy photosynthesis though individual leaf photosynthesis was high.

Dry bean yield was increased under shade in comparison to the full sun. Although the abortion of flowers and cherelles was higher in shade than in the full sun, the fact that the numbers were higher from the beginning means that number of pods ended up being higher. <2 % of the flowers produced developed into cherelles among all the treatments, thus confirming previous studies (de Almeida and Valle, 2007; Groeneveld et al., 2010; Carr and Lockwood, 2011). Though flower abortion and cherelle wilt may be inherent traits to manage resource allocation (Mckelvie, 1956; Handley, 2016), below cocoa canopy temperature in the full sun conditions were high which may have affected flower development to cherelles, especially in the water-suppressed plots. Stigma viability, pollination, pollen tube growth and early embryo development are vulnerable to heat stress (Giorno et al., 2013; Lamaoui et al., 2018). Lack of rainwater during anthesis and early cherelle development also cause abnormalities in floral organs, interfering with pollination and inducing abscission of newly formed embryos (Saini, 1997). In effect, Frimpong-Anin et al. (2014) observed a larger drop of unpollinated flowers in the dry season than in the rainy season. Pod damage in our study was, however, more pronounced under the shade than under the full sun contrary to previous studies (Ofori-Frimpong et al., 2007). Bos et al. (2006) observed that early and pathogenic fruit losses were more numerous under shade. Low temperature combined with increased humidity under shade could have provided a favourable environment for fungal diseases such as *Phytophthora* spp. causing damage to cherelles and pods (Delgado-Ospina et al., 2021). Still, the higher initial numbers of flowers, the heavier pods and beans under the shade compared to the full sun conditions resulted in an overall higher yield under shade. Additionally, pruning of cocoa trees may provide a more uniform light distribution within the canopy and allow better airflow, thus reducing moisture that could otherwise favour fungal development (Riedel et al., 2019; Delgado-Ospina et al., 2021).

Yield was sensitive to water availability and declined at increasing rainwater suppression, and bean quality declined in response to limited soil moisture. Similar studies of yield response to water availability have been reported by Abdulai et al. (2018) in West Africa, Gateau-Rey et al. (2018) in South America and Wuriandani et al. (2018) in Asia. Interactions between shade level and rainwater suppression level were significant for some of the variables, including soil water content, diurnal variations in water potential, WUE and F_v/F_m , mostly suggesting that effects of water stress were less pronounced under shade compared to full sun. This also appeared to be the case for canopy density, number of flowers and parameters related to pod dimensions and number of seeds. However, for variables such as photosynthesis, substomatal CO_2 concentration, predawn water potential, and most importantly the yield, interactions were not significant despite clear and significant effects of both rainwater suppression and shade level. Hence the effects of stress on these parameters are mainly additive. In contrast to our hypothesis, this leads to the interpretation that shade may not prevent cocoa yield from declining under drought stress. Thus, the overall better performance of cocoa under shade can only partly compensate for the reduced yield during drought episodes.

In the present study, yield increased under shade in contrast with some previous reports (Ahenkorah et al., 1974; Blaser et al., 2018;

Niether et al., 2020) where higher yields were observed under full sun than in agroforestry conditions. The question is whether our results can be translated directly to agroforestry. This study applied shade nets to provide uniform shade, but due to high costs, this is not an affordable shade management practice for many farmers. The alternative will be to apply natural shade from shade trees extending above the canopy of cocoa. There is an increasing body of evidence showing that shade trees may increase cocoa yields under conditions of low agricultural inputs (including fertilizers and pesticides) (Wouter et al., 2016; Andres et al., 2018; Sauvadet et al., 2019; Asare et al., 2019; Asitoakor et al., 2022). However, although such trees provide shade that will benefit the cocoa trees, they may also compete with cocoa for water and nutrients, as well as influencing the occurrence of pests and diseases (Blaser et al., 2018; Mortimer et al., 2018; Kaba et al., 2020;). Abdulai et al. (2018) showed that shade trees could have a negative impact on cocoa under severe drought stress conditions, and that such interactions depended on the shade tree species. There is a need to investigate how cocoa physiology and performance are influenced by water stress when under agroforestry conditions, and in particular, how they are influenced by various tree species in differing local conditions.

5. Conclusion

Overall, the experiment confirmed that shade has a positive impact on cocoa, not only enhancing yield but also the apparent health and growth of the cocoa trees. As expected, rainwater suppression led to drought and decreased performance of the cocoa trees in terms of physiological performances, growth, and yield. However, our expectation that shade would remedy the consequences of the drought was not unequivocally confirmed, as there were only a few interactions between shade and rainwater suppression, suggesting that the effects of the two factors were mainly additive. This means that even though shade will benefit the cocoa plants, it will not prevent them from being stressed by low soil water availability. Further research is needed to clarify how agroforestry shade tree species impact cocoa under drought, particularly regarding their root profile and ability to tap water at lower soil depths and enhancing water availability in the upper soil layers via hydraulic lift.

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CRediT authorship contribution statement

Eric Opoku Mensah: methodology, investigation, data collection, writing-original draft, formal analysis, **Anders Ræbild:** conceptualization, supervision, validation, methodology, writing-reviewing and editing, **Richard Asare:** conceptualization, resources, supervision, writing-reviewing and editing, **Christiana A. Amoatey:** supervision, project administration, writing- reviewing and editing, **Bo Markussen:** visualization, validation, formal analysis, **Kwadwo Owusu:** resources, funding acquisition, project administration, **Bismark Kwesi Asitoakor:** investigation, data collection, writing-reviewing and editing, **Philippe Vaast:** conceptualization, supervision, validation, methodology, writing-review and editing

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165657>.

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