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RESEARCH ARTICLE

High phosphorus supply enhances leaf gas exchange and growth of young Arabica coffee plants under water deficit

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Abstract

Drought is considered as the major environmental stress affecting coffee production, and high phosphorus (P) supply may alleviate the drought effects on crop metabolism. Here, we hypothesized that high P supply would mitigate the impacts of drought on Arabica coffee physiology, morphology, and biomass accumulation. Potted Arabica coffee plants were grown under two P levels: the recommended P fertilization (P), and twice the recommended fertilization (+P), and two water regimes: well-watered and water withholding for 32 days. Leaf, stem, and root P concentrations were increased under +P, with plants showing higher photosynthesis and growth than the ones receiving the recommended P dose. Higher plant growth under high P supply seems to upregulate leaf photosynthesis through the source–sink relationship. Under the water deficit, the reduction of leaf photosynthesis, stomatal conductance, transpiration, water use efficiency, carboxylation efficiency, chlorophyll content, number of plagiotropic branches, plant leaf area, and vegetative biomass production was similar comparing plants fertilized with the recommended P to those supplied with +P. However, Arabica coffee trees under high P supply and water deficit presented morphological and physiological traits similar to plants under well-watered and recommended P fertilization.

Keywords: Biomass; Chlorophyll; Photosynthesis; Starch; Sucrose; Water use efficiency

Introduction

The inter- and intra-annual precipitation patterns have changed in many regions of the world during the last decades (Sousounis and Little, 2017), and drought is considered as the major environmental stress affecting coffee production in several coffee-growing countries, including Brazil (Venancio *et al.*, 2020). In fact, coffee yield has been reduced up to 80% in very dry years in some marginal regions (DaMatta *et al.*, 2010), and such yield reduction is a consequence of drought impact on metabolism and growth of coffee trees (DaMatta *et al.*, 2019). The physiological cascade of responses to drought is often related to reduced water uptake and transport (Chaves *et al.*, 2002), stomatal closure (Cornic, 2000), and non-stomatal limitations to photosynthesis (Zhou *et al.*, 2013), leading to the inhibition of CO_2 uptake under prolonged drought period. However, alleviation of drought effects on plant metabolism by phosphorus (P) supply has been reported in annual crops such as common bean (Santos *et al.*, 2004) and soybean (Jin *et al.*, 2006), in C4 grasses (Kuwahara *et al.*, 2016), and in tree species, as *Alnus cremastogyne* (Tariq *et al.*, 2018).

Phosphorus is a plant macronutrient with major impact on crop productivity and carbon metabolism (Niinemets *et al.*, 2001) as four molecules of inorganic P (P_i) must enter the chloroplast for every molecule of sucrose synthesized in the cytosol (Sivak and Walker, 1986). In fact, the cytosolic P_i concentration controls the rate of photosynthesis and partitioning of photoassimilates between starch and sucrose (Rychter and Rao, 2005). While the scientific literature is rich when considering how low P supply affects plants (Partelli *et al.*, 2009; Meena *et al.*, 2021), little is known about how extra P supply may benefit plants under stressful conditions. Under non-limiting conditions, coffee plants receiving extra P supply (twice the recommended) presented enhanced plant hydraulic conductance, leaf carbohydrate, and chlorophyll contents, resulting in improved growth as compared with trees supplied with the recommended P fertilization (Silva *et al.*, 2010). Under field conditions, elevated P doses caused an increase in branch length and leaf area, number of fruits, and yield of coffee plants (Mera *et al.*, 2011), but the underlying physiological processes leading to improved performance remain unknown.

Coffee trees, as perennial, naturally experience seasonal water deficit, especially before flowering, which leads to impaired growth (Rakocevic and Matsunaga, 2018; Silva *et al.*, 2004) and low photosynthetic activity (Rakocevic *et al.*, 2018). A meta-analysis of drought impacts on various plant species revealed decreases in N and P uptake and impaired plant growth (He and Dijkstra, 2014). Herein, we hypothesized that young Arabica coffee plants supplied with high P doses would improve the photosynthetic performance and growth under drought conditions, partially mitigating the impacts of drought on coffee physiology and biomass accumulation. For testing that hypothesis, we evaluated how high P supply affected leaf gas exchange, photochemical activity, photosynthetic pigments, leaf carbohydrate availability, and morphological characteristics of young Arabica coffee plants exposed to long-term water deficit, which were additionally evaluated after a recovery period.

Material and Methods

Plant material and experimental conditions

Young plants of Arabica coffee (*Coffea arabica* L.) cv. Ouro Verde, obtained from self-pollination, were used in this study. Uniform 8-month-old seedlings with three pairs of leaves and around 0.12 m height were transplanted to plastic pots (9.1 dm^3). Just before seedling transplantation, soil adhered to roots was carefully removed to reduce nutrient availability. Plant recovery from transplantation was confirmed by vegetative growth and appearance of one new pair of leaves after 15 days. Then, these young coffee plants were grown under greenhouse conditions, where the maximum photosynthetic photon flux density (PPFD) was 1,600 $\mu mol\ m^{-2}\ s^{-1}$ and air temperature ranged between 26.4 and 36.2 °C. These two environmental variables were monitored with a quantum sensor model Li-190SB (Licor, Lincoln NE, USA) and an Onset Hobo datalogger model U12 (Onset, Bourne MA, USA).

The soil used in the experiment was collected from 0.0 to 0.2 m layer (bulk soil density of 1.2 kg dm^{-3}) with the following chemical composition: organic matter content (OM) 25 g dm^{-3}; pH ($CaCl_2$) 4.2; 2.0 mg P dm^{-3}; 1.1 $mmol_c$ K dm^{-3}; 7.0 $mmol_c$ Ca dm^{-3}; 3.2 $mmol_c$ Mg dm^{-3}; 0.26 mg B dm^{-3}; 2.0 mg Cu dm^{-3}; 55 mg Fe dm^{-3}; 3.0 mg Mn dm^{-3}; 0.6 mg Zn dm^{-3}; 5.0 $mmol_c$ Al dm^{-3}; sum of bases (SB) 11.0 $mmol_c$ dm^{-3}; cation exchange capacity (CEC, at pH 7) 50.7 $mmol_c$ dm^{-3}; and base saturation (V) 25% which was evaluated according to van Raij *et al.* (2001). Before planting, dolomitic lime (1.2 g dm^{-3} soil) was applied to increase V to 50%. Soil was fertilized as recommended for coffee plants, with pots receiving 72 mg N dm^{-3} (as urea) and 120 mg K dm^{-3} (as potassium chloride). The post-planting fertilization was performed with 3 g pot^{-1} of N and K in 30-day intervals. Fertilization with micronutrients was done with 0.6 mg B dm^{-3}, 5.7 mg Zn dm^{-3}, 6 mg Mn dm^{-3}, and 1.2 mg Cu dm^{-3} at 45 days after transplanting.

Phosphorus supply and water regimes

After transplantation, Arabica coffee plants were grown under two P levels: the recommended P fertilization (P), with 345 mg P_2O_5 dm^{-3} soil (Malavolta *et al.*, 1981), and twice the recommended fertilization (+P), with 690 mg P_2O_5 dm^{-3} soil, using monoammonium phosphate (MAP) as source. All plants were maintained well-watered (80% of soil water-holding capacity, SWC) for 3 months when the average leaf P concentration was 1.23 ± 0.15 and 1.87 ± 0.06 g kg^{-1} in P and +P plants, respectively (Silva *et al.*, 2010). Plants from both treatments were well supplied with phosphorus, but P plants reached intermediate P concentration in leaves (from 1.3 to 1.5 g kg^{-1}), while +P plants presented high P status (> 1.5 g kg^{-1}). Then, one group of plants from each P treatment was subjected to water withholding (D), while the other was maintained under 80% SWC (W). Thus, four conditions (treatments) were established: P under 80% SWC (PW); P under drought (PD); +P under 80% SWC (+PW); and +P under drought (+PD). The maximum water deficit occurred when the plants presented significant leaf wilting at early morning, which happened after 32 days of water withholding. Then, soil was rehydrated for 4 days, and the recovery capacity of plants based on leaf gas exchange was evaluated. At the end of the experiment, soil samples were collected, and chemical analyses performed as described earlier.

Leaf water status

The leaf water potential (Ψ_w) was evaluated at predawn (5h00–6h00) with a pressure chamber model 3005 (Soilmoisture Equipment Corp., Santa Barbara CA, USA). In leaves close to ones used for evaluating gas exchange, the Ψ_w was measured at the 1st, 16th, 27th, and 32nd day of water withholding and at the 1st and 4th day of recovery, that is, the 33rd and 36th day of experiment, respectively.

The relative water content (RWC) was determined at the 32nd day of water withholding (maximum water deficit), using leaf disks with 1 cm diameter. RWC was defined as: RWC(%)=[(FM-DM)/(TM-DM)]*100, where FM, DM, and TM are the fresh, dry, and turgid (after 24 h immersed in pure water under PPFD of 50 μmol m^{-2} s^{-1}) mass of leaf disks, respectively (Weatherley, 1950).

Leaf gas exchange and chlorophyll fluorescence

While leaf gas exchange was measured whenever Ψ_w was evaluated, the chlorophyll fluorescence measurements were taken only at the maximum water deficit. Gas exchange and chlorophyll fluorescence were measured in the newest fully expanded leaves, using an infrared gas analyzer Li-6400 (Licor, Lincoln NE, USA) and a modulated fluorometer 6400-40 LCF (Licor, Lincoln NE, USA), respectively. We evaluated the net photosynthetic rate (A), stomatal conductance (g_s), transpiration (E), and intercellular CO_2 concentration (C_i). The intrinsic water use efficiency (iWUE = A/g_s) and the instantaneous carboxylation efficiency (iCE = A/C_i) were then estimated. The effective quantum efficiency of PSII (ϕ_{PSII}) and the photochemical (q_P) and non-photochemical (NPQ) quenching of fluorescence were also evaluated in light-adapted leaves, as done previously (Silva *et al.*, 2010). Measurements were taken under PPFD of 1,200 μmol m^{-2} s^{-1} and air CO_2 partial pressure of 38 Pa, between 9h00 and 11h00, after data reaching temporal stability and low total coefficient of variation (<2.5%). Along the experimental period and during measurements, the average leaf-to-air vapor pressure deficit (VPD_L) varied between 0.95 and 2.43 kPa and leaf temperature between 25.9 and 34.8°C, both measured with the Li-6400.

Pigments

Leaf chlorophyll (Chl) *a* and *b* and total carotenoid (Car, given by carotenes and xanthophylls) were evaluated at the maximum water deficit and 4 days after rewatering (recovery). Pigment extraction was performed in leaf disks (1 cm diameter) with acetone solution (80%, v/v), using a mortar and pestle, under dark conditions. The extract was centrifuged, and the absorbance of supernatant was measured at 470, 646, and 663 nm with a spectrophotometer. Pigment concentrations were calculated according to Lichtenthaler and Wellburn (1983), and the total Chl content was estimated as the sum of Chl *a* and Chl *b* contents.

Leaf carbohydrates

Leaves nearby to those ones used in gas exchange measurements were collected at the maximum water deficit and 4 days after rewatering (recovery). The leaf samples were lyophilized (Freezone 4.5, Labconco, Kansas City MO, USA) and then ground. The soluble carbohydrate extraction was done with a solution of methanol:chloroform:water (15:5:3 v/v) for 72 h at 4 °C (Bieleski and Turner, 1966). The supernatant was used for determining the content of soluble sugars and sucrose, which were evaluated by the phenol-sulfuric acid method following Dubois *et al.* (1956) and van Handel (1968), respectively. Starch content was determined in the precipitate (insoluble fraction) after extraction of soluble carbohydrates, following the enzymatic method proposed by Amaral *et al.* (2007).

Plant growth and biomass

Morphological evaluations were performed at the end of the recovery period. Plant height was measured from the stem basis to the top apex with a graduated ruler. Stem diameter was measured near the soil surface with a digital caliper rule (model 100.179G, Digimess, São Paulo SP, Brazil). The number of leaves and plagiotropic branches were counted in each plant. The leaf area (LA, m^2 $plant^{-1}$) was determined with a digital planimeter (Li-3000C, Licor Inc., Lincoln NE, USA). All plants were fractioned in leaves, stem (including the main stem), and roots, and the fractions were dried in an oven (65 °C) until constant weight. Then, leaf, stem, and root dry mass (DM) were determined, and the leaf mass per area (LMA, mg cm^{-2}) was calculated as the ratio between leaf DM and LA.

Nutritional analysis

After the recovery period, that is, at the 37th day of experiment, leaf, stem, and root dry samples were ground and passed through a sieve with a mesh diameter of 1 mm. After a previous wet digestion with nitric acid and/or percloric acid, the concentration of N was determined by distillation and the other nutrients (P, K, S, Ca, Mg, B, Cu, Fe, Mn, and Zn) by inductively coupled plasma optical emission spectrometry, as described by Bataglia *et al.* (1983).

Experimental design and data analyses

The experimental design was completely randomized with four treatments resulting from the combination of two factors: phosphorus availability (P and +P) and water conditions (W and D), referred to as PW, +PW, PD, and +PD. The number of replications varied from 3 to 5, depending on the variable evaluated. The experimental unit was a pot with one plant. Data were subjected to the analysis of variance (ANOVA), after testing the hypothesis of variance homogeneity among treatments by Levene's test (Levene, 1960). Whenever variances were heterogeneous, Welch's correction was applied (Welch, 1951). Differences among treatment means or factor level means were evaluated by using F-tests for contrasts at 0.05 significance level (Box *et al.*, 2005). For variables measured at more than one time, analyses were done by time. ANOVA was performed using SAS/STAT (SAS Institute Inc., 2003) procedures, GLM or MIXED, for cases with homogeneous or heterogeneous variances, respectively.

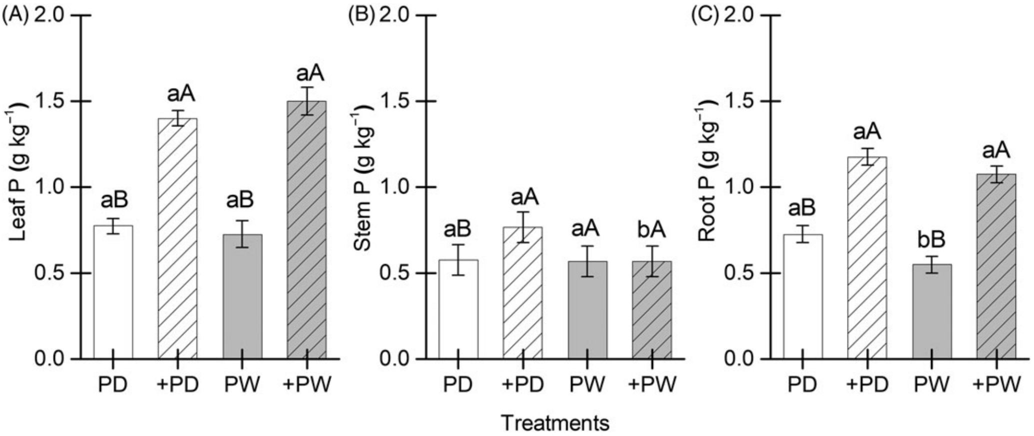

Figure 1. Phosphorus concentration in leaves (A), branches (B), and roots (C) of young Arabica coffee plants receiving recommended P fertilization (P) or high P supply (+P) and grown under well-watered (W) or water-deficit (D) conditions. Samples were taken at the end of the experiment. Vertical lines correspond to standard errors of mean estimates ($n = 4$). Small letters compare water regimes for a given phosphorus level, and capital letters compare phosphorus levels for a given water regime.

Results

Soil conditions

Some soil characteristics differed at the end of experiment compared to the initial conditions, as shown in Supplementary Material Table S1 available online at https://doi.org/10.1017/S0014479722000266. While pH ($CaCl_2$) ranged from 4.1 to 4.2 and was not changed, levels of K, Ca, Cu, Mn, Al, CEC, and V increased as compared with sampling done before the fertilization. Among treatments, soil P, Fe, and Zn contents were increased while OM, Mg, and SB were reduced under high P supply as compared with P treatment, regardless of water regime (Table S1). When considering water regimes under high P supply, soil B and Fe contents were higher under well-watered conditions as compared with water-deficit treatment. When pots faced water deficit, soil Zn and P contents were increased under high P supply (Table S1).

Plant nutritional status

At the end of experimental period, leaf, stem, and root P concentrations were affected by P supply and water conditions (Figure 1; Supplementary Material Table S2). Leaf P concentration had not been impacted by water availability but increased significantly with high P supply (Figure 1A). The average P values were reduced over the experimental period – likely due to a dilution effect caused by plant growth, that is, from 1.87 to 1.50 g kg^{-1} in +P plants and from 1.23 to 0.75 g kg^{-1} in P plants (Figure 1A). High P supply increased stem P concentration under drought (Figure 1B), while root P concentration was increased under high P supply, regardless of water regime. However, drought increased root P concentration only under recommended P supply (Figure 1C).

High P supply reduced stem concentrations of Ca, Mg, S, B, Cu, Fe, and Mn and leaf concentrations of N, Ca, and Cu (Supplementary Material Table S3). Only leaf S concentration increased due to high P supply as compared with recommended P fertilization. In roots, the opposite trend in relation to P supply was noticed, with increases in concentrations of N, K, Ca, S, and Zn. Water deficit increased stem N concentration and reduced leaf N, S, and Cu under high P supply, while increasing leaf Ca concentrations when plants received the recommended P doses (Table S3). Water deficit also increased root K, Ca, Mg, and S concentrations under recommended P fertilization, and S and Mn under high P supply.

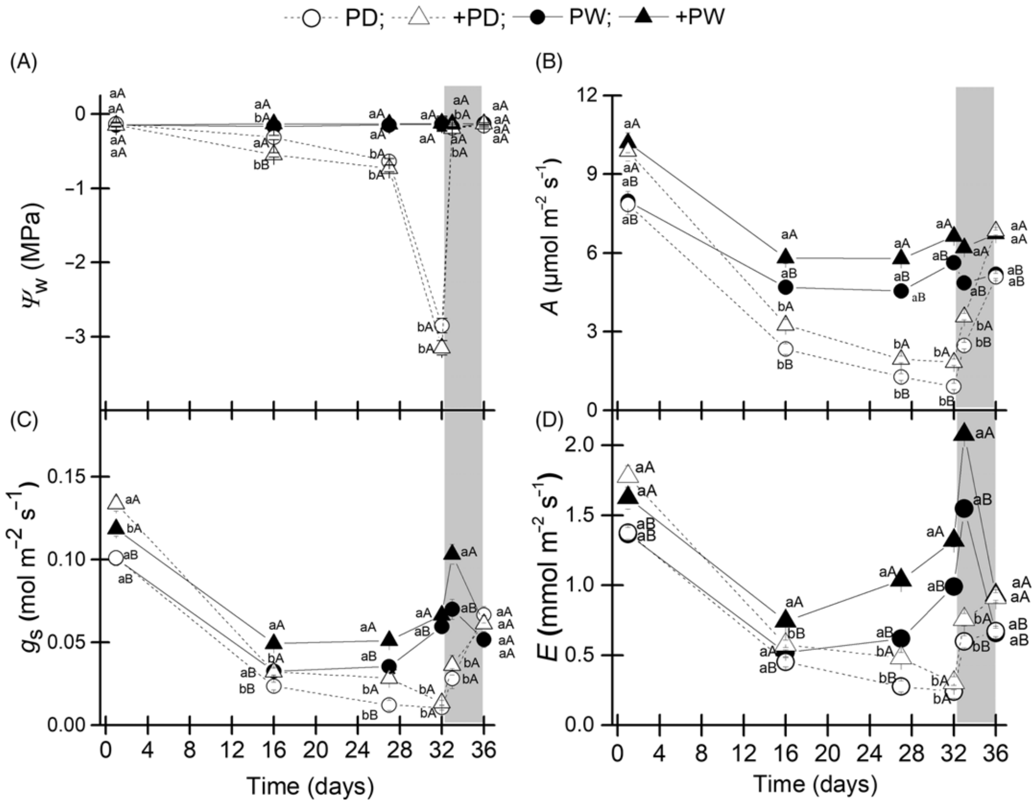

Figure 2. Temporal dynamics of leaf water potential (ψ_w, in A), stomatal conductance (g_s, in B), leaf CO_2 assimilation (*A*, in C), and transpiration (*E*, in D) of young Arabica coffee plants receiving recommended P fertilization (P) or high P supply (+P) and grown under well-watered (W) or water-deficit (D) conditions. The maximum water deficit was reached after 32 days of water withholding and then plants were rehydrated for 4 days, that is, recovery period (gray area). Vertical lines correspond to standard errors of mean estimates ($n = 3–4$). In each evaluation time, small letters compare water regimes for a given phosphorus level and capital letters compare phosphorus levels for a given water regime.

Plant water status

Ψ_w was reduced after 16 days of water withholding, regardless of P supply (Figure 2A). The lowest Ψ_w values were reached after 32 days of water deficit, and there was no mitigation effect of high P supply on leaf water potential. At the maximum water deficit, leaf RWC was reduced from 95 to 73% (PD) and 80% (+PD) due to low water availability, and again P supply did not induce differential response (Supplementary Material Figure S1). Ψ_w was increased in plants previously exposed to drought just after 1 day of soil rewatering, and full recovery Ψ_w was noticed after 4 days (Figure 2A).

Leaf gas exchange, photochemistry, and pigments

The highest values of g_s, *A*, and *E* along the experimental period were found in coffee plants under high P supply (Figure 2B-D). When plants were subjected to water deficit, decreases in leaf gas exchange were noticed and the lowest values of *A*, g_s, and *E* were recorded after 27–32 days of water withholding. However, plants under high P supply exhibited higher *A*, g_s, and *E* than ones receiving the recommended P amount during the water-deficit period (Figure 2B-D). One day after soil rewatering was not enough for total recovery of *A* and *E*, which occurred after 4 days of rewatering and remained higher in plants under high P supply (Figure 2C,D). Stomatal aperture

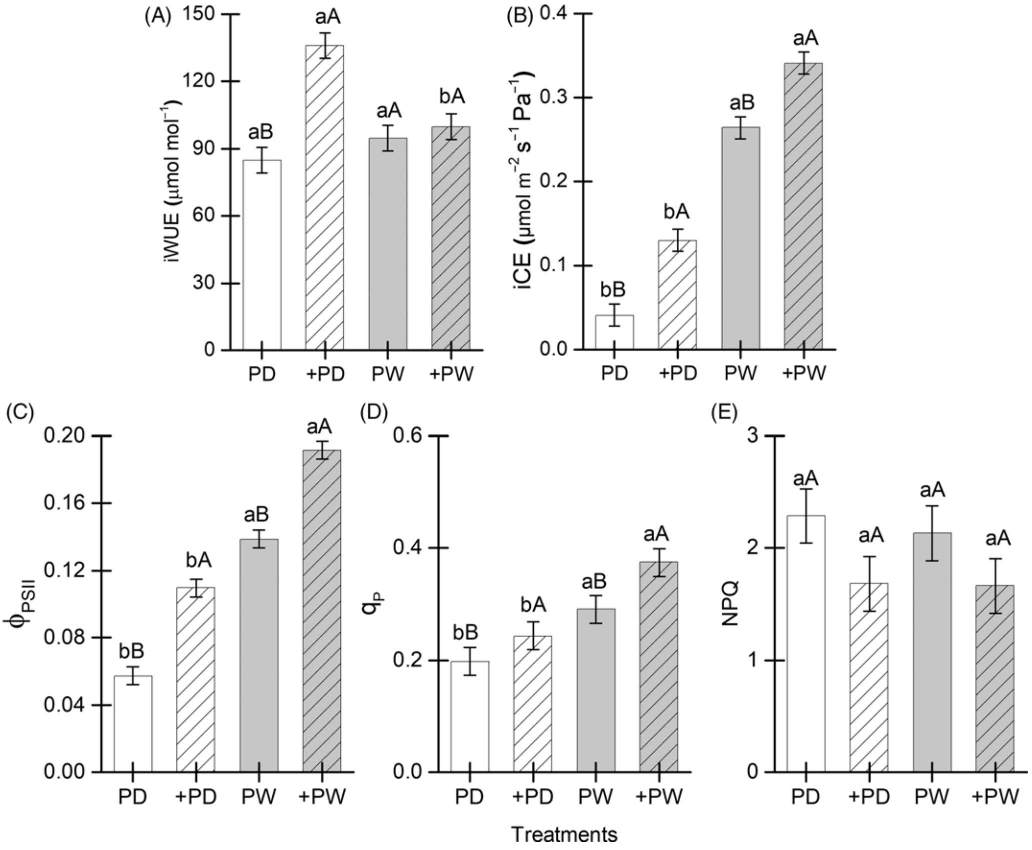

Figure 3. Water use efficiency (iWUE, in A), instantaneous carboxylation efficiency (iCE, in B), effective quantum efficiency of PSII (ϕ_{PSII}, in C), photochemical quenching (q_P, in D) and non-photochemical quenching (NPQ, in E) of young Arabica coffee plants receiving recommended P fertilization (P) or high P supply (+P) and grown under well-watered (W) or water-deficit (D) conditions. Measurements were taken at the maximum water deficit. Vertical lines correspond to standard errors of mean estimates ($n = 3$). Small letters compare water regimes for a given phosphorus level and capital letters compare phosphorus levels for a given water regime.

was also recovered after 4 days of rewatering, with plants from all treatments exhibiting similar values (Figure 2B).

At the maximum water deficit, iWUE was increased due to low water availability only under high P supply (Figure 3A, Table S2). Water deficit reduced iCE, ϕ_{PSII}, and q_P in both P treatments, but plants under high P supply always presented higher iCE, ϕ_{PSII}, and q_P than ones supplied with the recommended P doses, regardless of water condition (Figure 3B-D). NPQ was not changed either by water conditions or P supply (Figure 3E).

Chl *a*, *b*, and $a + b$ concentrations were reduced under drought in both P treatments at the maximum water deficit (Figure 4A-C). High P supply increased Chl *b* and then Chl $a + b$ concentrations as compared to ones under recommended P fertilization, regardless of water regime (Figure 4B-C). On the other hand, high P supply reduced leaf carotenoid concentrations, especially under water deficit (Figure 4D). After 4 days of rehydration, only plants under high P supply recovered leaf Chl *a* concentration (Figure 4E). Chl *b*, Chl $a + b$, and carotenoid contents were also recovered and were similar between water regimes (Figure 4F-H). However, high P supply caused higher concentrations of Chl *a*, Chl *b*, and Chl $a + b$ after rehydration than the treatment with recommended P fertilization, regardless of water regime (Figure 4E-G).

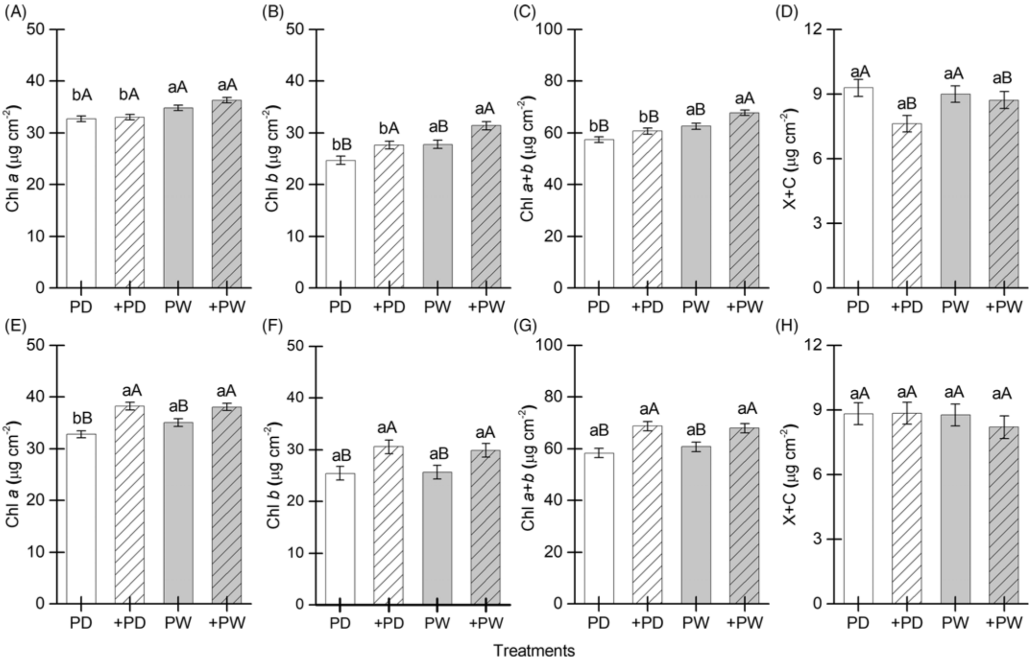

Figure 4. Leaf concentrations of chlorophyll a (Chl *a*, in A, E), chlorophyll b (Chl *b*, in B, F), Chl $a + b$ (in C, G), xanthophylls and carotenes (X + C, in D, H) of young Arabica coffee plants receiving recommended P fertilization (P) or high P supply (+P) and grown under well-watered (W) or water-deficit (D) conditions. Measurements were taken at the maximum water deficit (A-D) and after 4 days of rehydration (E-H). Vertical lines correspond to standard errors of mean estimates ($n = 4$). Small letters compare water regimes for a given phosphorus level and capital letters compare phosphorus levels for a given water regime.

Leaf carbohydrates

At the maximum water deficit, concentrations of starch, sucrose, and soluble sugars were reduced in leaves of coffee plants supplied with the recommended P fertilization (Figure 5A-C). Such reductions were also found for sucrose and soluble sugars in plants under high P supply (Figure 5B-C). Overall, the amount of total non-structural carbohydrates (NSCs) was reduced by water deficit, regardless of P fertilization (Figure 5D). However, coffee plants under high P supply presented lower NSC than ones under recommended P fertilization at the maximum water deficit (Figure 5D). After 4 days of rewatering, the leaf starch content remained lower in plants previously exposed to drought, and the lowest values were found in plants under high P fertilization (Figure 5E). Leaf concentrations of sucrose, soluble sugars, and NSC were recovered only in plants under high P supply, while the opposite was found for plants receiving the recommended P amount (Figure 5F-H).

Plant growth

Water deficit reduced plant growth and negatively affected all morphological variables, regardless of P supply (Figure 6). However, high P supply increased plant height (data not shown), trunk diameter, number of plagiotropic branches, number of leaves, and leaf area, resulting in increased leaf, stem, and root biomass and reduced root/shoot ratio (Figure 6). Coffee plants allocated more biomass to roots under drought, whereas allocation to leaves was prioritized under high P supply (Figure 6D). LMA was not modified by either P supply or water availability, varying between 5.11 and 5.39 mg cm^{-2}.

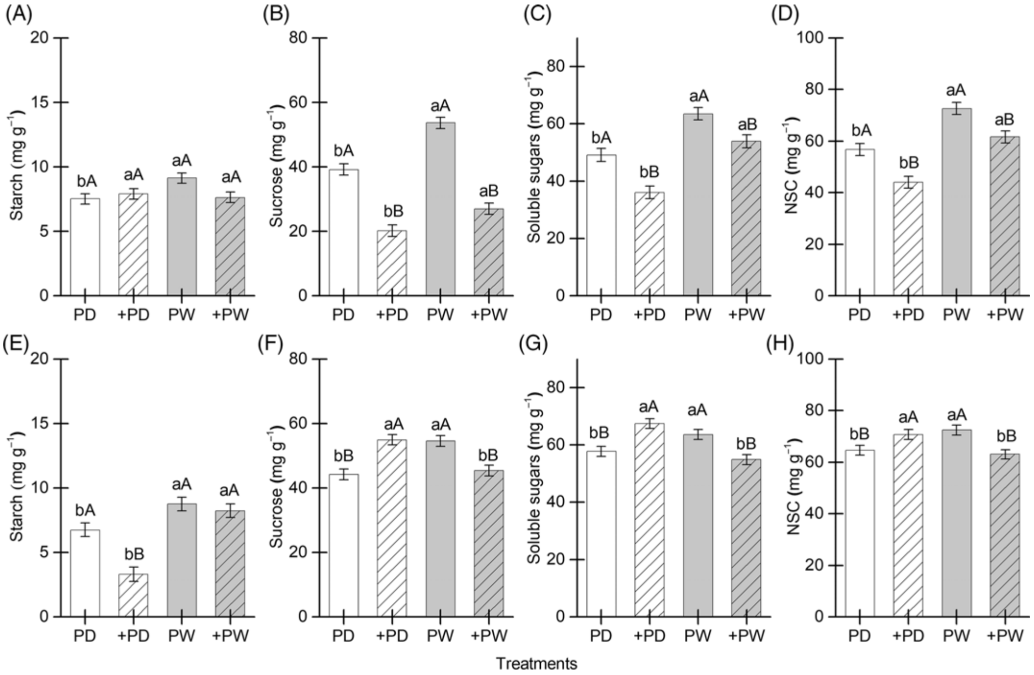

Figure 5. Leaf concentrations of starch (A, E), sucrose (B, F), soluble sugars (C, G), and non-structural carbohydrates (NSC, in D, H) of young Arabica coffee plants receiving recommended P fertilization (P) or high P supply (+P) and grown under well-watered (W) or water-deficit (D) conditions. Measurements were taken at the maximum water deficit (A-D) and after 4 days of rehydration (E-H). Vertical lines correspond to standard errors of mean estimates ($n = 3$). Small letters compare water regimes for a given phosphorus level and capital letters compare phosphorus levels for a given water regime.

Discussion

The overall effects of water deficit on coffee physiology have been explored elegantly during the last decades, and our results about drought effects agree with the current literature (DaMatta *et al.*, 2010, 2019; Menezes-Silva *et al.*, 2017; Silva *et al.*, 2004). Precisely, this paper was dedicated to understanding how high P supply would benefit young Arabica coffee plants under water deficit. Despite plant morphology being more conservationist than rapid leaf gas exchange responses, there is a general close dependence between plant structure and function (Vos *et al.*, 2010), as noticed herein. As a key outcome from our research, we found that high P supply improved Arabica coffee growth and leaf gas exchange, but contrary to what we expected, such positive effects were noticed regardless of water availability (Figures 2, 3 and 6). The leaf, stem, and root nutritional status changed under drought conditions (Figure 1), and plants facing water deficit and supplied with the recommended P doses invested more P in roots, while plants under high P supply invested more P into stems (Figure 1). The role of arbuscular mycorrhizal fungi on plant nutrition (Begum *et al.*, 2020) would explain such differential P partitioning in coffee trees under varying P fertilization, a subject to be further explored in future research.

While at the beginning of the experimental period, the Arabica coffee plants receiving the recommended P dose had leaf P status indicating sufficiency (Malavolta, 1981; Reis *et al.*, 2011), they ended the experiment with leaf P concentration lower than 1.0 g kg^{-1}. Besides the dilution effect previously commented (Results section), one cannot rule out any influence of water deficit on P uptake when water was unavailable. Even with plants under recommended P fertilization showing low P status, our physiological indices and the visual aspect of plants did not suggest any P deficiency.

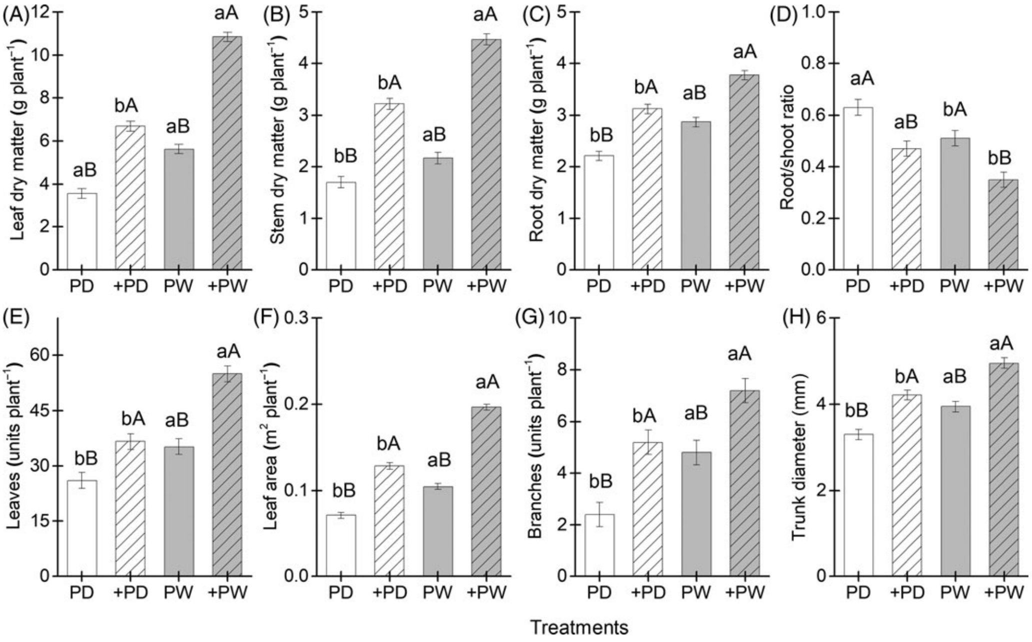

Figure 6. Leaf (A), branch (including trunk, B) and root (C) dry matter, root/shoot ratio (D), number of leaves (E), leaf area (F), number of plagiotropic branches (G), and trunk diameter (H) of young Arabica coffee plants receiving recommended P fertilization (P) or high P supply (+P) and grown under well-watered (W) or water-deficit (D) conditions. Measurements were taken at the end of the experiment. Vertical lines correspond to standard errors of mean estimates ($n = 5$). Small letters compare water regimes for a given phosphorus level and capital letters compare phosphorus levels for a given water regime.

Overall, the responses of Arabica coffee plants to water deficit were similar for most morpho-physiological variables when comparing plants supplied with the recommended P amount to those supplied with high P (Figures 2 to 6). Even with water deficit reducing significantly plant performance, coffee plants under high P supply and water deficit exhibited growth similar or higher than plants under recommended P fertilization and well-watered conditions (Figure 6). This was the main novelty of this paper, and additional questions about coffee nutrition can be formulated: has phosphorus demand by coffee trees increased with breeding? While it is reasonable to assume that more productive coffee cultivars would need more nutrients to compensate the dilution effect caused by increased biomass production and yield, one should consider that cultivars differ in coffee yield and growth and then their demand for nutrients would differ as well. As an example, Teixeira *et al.* (2015) reported a large variation in crop yield when comparing coffee cultivars, with green Arabica coffee production varying from 23.1 (cv. Bourbon Amarelo UFV 535) to 43.5 bags ha^{-1} (cv. Catuaí Vermelho IAC 15). Changes in coffee fertilization through decades were clearly shown by Malavolta (1981), with P_2O_5 fertilization moving from 35 g/plant/year in early 1890s to 100 g/plant/year in 1970s. Even with coffee breeding programs launching cultivars adapted to soils with low P content and with high P use efficiency (Neto *et al.*, 2016), we found a positive response to high P supply, regardless of water availability.

About our initial hypothesis, high P supply alleviated the impact of water deficit on leaf carbohydrate status during the recovery period. In plants previously exposed to water deficit, the availabilities of sucrose, soluble sugars, and then NSC in leaves were increased under high P supply. Such increases in soluble carbohydrates were a likely consequence of enhanced starch degradation in plants receiving high P supply (Figure 5E-H). In wheat, P supply has been reported to induce genes linked to starch degradation, increasing the transcript abundance of amylases (Zhang *et al.*, 2018). If such phenomenon occurs in coffee plants, it seemed to be enhanced during the recovery of water deficit, when plants need to reestablish the pool of soluble sugars to resume growth. Further experimentation would reveal the molecular bases of such differential performance induced by high P supply during plant rehydration.

High P supply increased the water use efficiency of Arabica coffee plants at the maximum water deficit, and this was caused by high leaf photosynthetic rates rather than decreases in leaf transpiration (Figures 2 and 3A). In fact, stomatal closure due to low leaf water status is a common and initial response to water deficit, affecting both leaf transpiration and photosynthesis (Chaves *et al*., 2002), as found herein (Figure 2A,B). However, higher g_s in Arabica coffee plants under high P supply would be a consequence of higher water uptake by a larger root system, as compared with plants under recommended fertilization (Figures 2 and 6). In this way, lower Ψ_w of plants under high P fertilization is likely caused by high leaf transpiration (Figure 2A,D).

A progressive inhibition of carboxylation becomes dominant under severe drought (Bota *et al*., 2004), and our data revealed that carboxylation rate was higher in Arabica coffee plants under high P supply, regardless of water availability. When facing water deficit, plants under high P availability presented iCE about four times higher than ones receiving the recommended P fertilization (Figure 3B). The higher carboxylation efficiency would be a consequence of changes in source–sink relationship under high P supply and their effects on expression of genes related to C_3 photosynthetic pathway (Paul and Pellny, 2003). In fact, citrus photosynthesis is stimulated by vegetative growth, which demands energy carbon skeletons for new shoot formation (Ribeiro *et al*., 2012). Similarly, one would argue that enhanced coffee plant growth (Figure 6) under high P supply would upregulate leaf photosynthesis (Figures 2C and 3B-D) through the source–sink relationship. Evidence supporting this assumption was the low level of soluble sugars during the maximum water deficit, that is, active sink caused decreases in available carbohydrates even when coffee plants showed high source activity under high P supply compared to recommended P fertilization (Figure 5B-D). During the recovery period, the higher P availability favored sucrose synthesis, using either recent photoassimilates or hexoses from starch degradation – as indicated by leaf carbohydrate dynamics (Figure 6E,F).

In common beans, leaf P spraying increased photosynthesis of plants under water deficit when nutrient uptake is impaired (Santos *et al*., 2004). Here, we found enhanced plant growth and physiological responses due to increasing soil P availability, with Arabica coffee trees showing higher photosynthetic rates supported by higher stomatal conductance, photochemical activity, and carboxylation efficiency (Figures 2, 3 and 6). From a practical perspective, high P supply increased the number of plagiotropic branches – the productive ones in adult plants, and this would justify increased Arabica coffee production when field-grown trees were supplied with high P doses (Mera *et al*., 2011).

Conclusions

Our initial hypothesis was partly confirmed, as the high P supply improved not only the performance of young Arabica coffee trees under water deficit but also of those maintained under well-watered conditions. The responsiveness of Arabica coffee trees to water deficit was not changed by P supply. However, coffee trees were favored by high P supply, presenting higher biomass accumulation as compared to plants receiving the recommended P doses. Coffee plants under high P supply and drought presented morphological and physiological performance like well-watered ones under recommended P fertilization, indicating that extra P supply is a way to reduce the impact of drought on Arabica coffee trees.

Supplementary Material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0014479722000266

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Competing Interests. The authors declare none.

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