

RESEARCH PAPER

Functional analysis of the relative growth rate, chemical composition, construction and maintenance costs, and the payback time of *Coffea arabica* L. leaves in response to light and water availability

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Abstract

In this study, the combined effects of light and water availability on the functional relationships of the relative growth rate (RGR), leaf chemical composition, construction and maintenance costs, and benefits in terms of payback time for *Coffea arabica* are presented. Coffee plants were grown for 8 months in 100% or 15% full sunlight and then a four-month water shortage was implemented. Plants grown under full sunlight were also transferred to shade and vice versa. Overall, most of the traits assessed were much more responsive to the availability of light than to the water supply. Larger construction costs (12%), primarily associated with elevated phenol and alkaloid pools, were found under full sunlight. There was a positive correlation between these compounds and the RGR, the mass-based net carbon assimilation rate and the carbon isotope composition ratio, which, in turn, correlated negatively with the specific leaf area. The payback time was remarkably lower in the sun than in shade leaves and increased greatly in water-deprived plants. The differences in maintenance costs among the treatments were narrow, with no significant impact on the RGR, and there was no apparent trade-off in resource allocation between growth and defence. The current irradiance during leaf bud formation affected both the specific leaf area and leaf physiology upon transferring the plants from low to high light and vice versa. In summary, sun-grown plants fixed more carbon for growth and secondary metabolism, with the net effect of an increased RGR.

Key words: Alkaloids, chemical composition, coffee, construction costs, growth, payback time, phenols, photosynthesis, sun and shade leaves, water deficit.

Introduction

Although carbon fixation, a process inherently associated with biomass construction, has been exhaustively investigated, very little research has been based on the subsequent fate of the carbon (C) that is assimilated. Indeed, the factors controlling plant respiration and the translocation of C to various organs are only partially understood (Poorter and Villar, 1997). In most studies, plant growth is simply considered as the accumulation of biomass, without considering the chemical composition of the constructed biomass. However, the way that a plant invests its photoassimilate

Abbreviations: AA, amino acids; ALK, alkaloids; A_m , mass-based net carbon assimilation rate; C, carbon; CC, construction costs; CC_P , the CC according to Poorter (1994); CC_W , the CC according to Williams *et al.* (1987); CV, coefficient of variation; DW, dry weight; FAD, fibres insoluble in acid detergent; FC, field capacity; FND, fibres insoluble in neutral detergent; H_c , heat of combustion; HCE, hemicellulose; HH, plants continuously grown under high light; HL, plants initially grown under high light and then transferred to low light; LH, plants initially grown under low light and then transferred to high light; LL, plants continuously grown under low light; LIP, lipids; MC, maintenance costs; MIN, minerals; N, nitrogen; N_{org} , organic N; OA, organic acids; PAR, photosynthetically active radiation; PCA, principal component analysis; PBT, payback time; PRO, proteins; RGR, relative growth rate; R_m , mass-based dark respiration rate; SLA, specific leaf area; STA, starch; TNC, total non-structural carbohydrates; TSS, total soluble sugars; WD, water deficit; $\delta^{13}C$, carbon isotope composition ratio.

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and the absorbed minerals into different chemical compounds directly influences its growth performance and construction (CC) and maintenance (MC) costs (Merino *et al.*, 1984; Villar *et al.*, 2006). The CC are defined by the amount of glucose used for constructing one gram of biomass (Penning de Vries *et al.*, 1974; Williams *et al.*, 1987) and typically range from approximately 1.1 to approximately 1.9 g glucose g⁻¹ for leaves of different species (Penning de Vries *et al.*, 1974; Sims and Percy, 1994; Poorter *et al.*, 1997, 2006; Poorter and Villar, 1997; Villar *et al.*, 2006). The CC are indirectly related to the relative growth rate (Williams *et al.*, 1987) and can be used to assess differences in resource use efficiency among plant species (Nagel and Griffin, 2001). The energy required to maintain processes that are unrelated to biomass gain, for example, the turnover of organic molecules, membrane maintenance, and solute exchange, is associated with the MC (Merino *et al.*, 1984). Low MC are generally associated with long-lived and sclerophyllous leaves and high amounts of structural components (Zhu and Cao, 2010).

Information on the effects of environmental factors, such as light, on the CC of woody species is conflicting. For example, Sims and Percy (1994) have estimated higher CC in the leaves of *Alocasia macrorrhiza* under low irradiance, whereas, in a study with 12 woody species, Poorter *et al.* (2006) have reported that leaves developed under low irradiance had, on average, lower (3%) CC than leaves exposed to high light intensities. In any case, only small or insignificant variations in CC have usually been reported in response to changes in environmental factors, supporting the hypothesis that alterations in the CC in response to such factors are relatively modest or non-existent (Poorter *et al.*, 2006). The pattern of correlation among leaf chemical constituents, either positive correlations among compounds with high and low energy costs or negative correlations among compounds of high energy costs, has been invoked to explain the small variations in CC observed in species growing in different ecosystems (Villar and Merino, 2001; Villar *et al.*, 2006). This pattern suggests that the chemical composition may vary greatly without any corresponding changes in the CC. In any case, it is not just the total CC, but the relative costs and benefits of an investment in any particular structure that should be considered (Karagatzides and Ellison, 2009). In this context, the payback time (PBT), which can be considered as the time span that a leaf must photosynthesize to recover (amortize) the carbon investment used in its construction, plays a special role because the PBT can be regarded as a measure of energy use efficiency, reflecting the plant energetic benefit during the leaf lifespan (Poorter *et al.*, 2006).

The hypothesis of compromise between growth and defence (survival) can explain the patterns of the chemical composition of a leaf (Rhoades and Cates, 1976; Coley, 1988). According to this theory, the plant faces an energetic choice of whether to synthesize defence compounds (with a high metabolic cost) or to invest that energy in growth-

related processes (Coley *et al.*, 1985; Westoby *et al.*, 2002). In this context, growth may compete with survival for common substrates, as is the case of phenylalanine, a precursor used in the synthesis of both phenolics and proteins. However, few studies have examined how the development of chemical defences can interact with leaf functional traits, considering the potential negative effects on growth and plant performance due to the diversion of resources for defence (Agrawal and Fishbein, 2006; Mondolot *et al.*, 2008; Pujol *et al.*, 2008).

Previous investigations on leaf chemical composition, CC, and PBT have often focused on plants grown entirely at high or low irradiance. However, in practice, the close spatial planting of crops always leads to a weak light environment around the lower mature leaves, whereas the newly developed leaves are expanded under high light (Jiang *et al.*, 2011). Because it has been found that the anatomical structure and photosynthetic capacity in the newly-developed leaves of some species are regulated by a systemic irradiance signal originating in the mature leaves (Lake *et al.*, 2001; Jiang *et al.*, 2011), it was hypothesized that the chemical composition and C economy in developing leaves would also depend on the light environment around the mature leaves rather than exclusively around the developing leaves per se.

Despite being native to shade environments, modern coffee (*Coffea arabica* L.) cultivars grow well without shade and even out-yield shaded coffee plants (DaMatta, 2004). The coffee tree exhibits high concentrations of secondary metabolic compounds, which are C-rich (e.g. phenols: Salgado *et al.*, 2008) and N-rich (e.g. methylxanthine alkaloids, particularly caffeine: Barros *et al.*, 1994; Ashihara *et al.*, 2008). Such compounds have generally been associated with an improved defence ability against herbivores, yet they also act against oxidative damage (phenols), thus, enhancing the survivability of the plant, even at the expense of growth (Close *et al.*, 2003). It is, therefore, suggested that the diversion of assimilate to produce high amounts of secondary compounds should greatly affect the C economy and chemical composition, which, in turn, should impact growth rates, although no quantitative approach has thus far been undertaken to examine these relationships in coffee.

To the best of our knowledge, information on the combined effects of light and water supplies on the CC, MC, and PBT is completely lacking. In the present study, the functional relationships among growth, leaf chemical composition, CC, and MC, and the benefits in terms of the PBT, as affected by environmental (light and water) factors, were examined as a means of increasing the understanding of the ecophysiological significance of the acclimation of coffee to light and water availabilities. In addition, it was also analysed whether the chemical composition, CC, MC, and PBT are affected by the light environment of mature leaves in a controlled factorial experiment where individuals, grown under full sunlight, were transferred to shade and vice versa and then these light treatments were combined with two water supplies.

Materials and methods

Plant material, growth conditions, and experimental design

The experiment was conducted in Viçosa (20°45' S, 42°54' W, 650 m in altitude) in south-eastern Brazil. To analyse the environmental variations exclusively and to avoid potential confounding sources, specifically genetic variation and variation associated with genotype×environment interactions, a single genotype (*Coffea arabica* L. cv 'Catuai Vermelho IAC 44') was studied. Uniform seedlings obtained from seeds were grown in 30 l pots containing a mixture of soil, sand, and composted manure (4:1:1, by vol.). There was no apparent restriction on root development as judged by examination at the end of the experiment. Sixty seedlings were planted in February 2009; half the plants were grown in high light, that is, full sun conditions, and the other half were grown under low light in a shade environment, that is, ~15% of full sunlight. The shade enclosure was constructed using neutral-density black nylon netting, and the plants were kept in these conditions for 8 months, after which 10 plants of each light treatment were harvested and characterized. Of the remaining 20 individuals growing under full sun, half remained under full sun, and the other half were transferred to the shade environment; the two light levels were then combined with two levels of available soil water (water deficit, WD, and field capacity, FC, defined here as 30% and 100% of available water, respectively—see below). The same scheme was carried out with the remaining 20 individuals growing in the shade, that is, half of the plants remained in the shade, and the other half were transferred to full sun, combining these light treatments with the two levels of available water. These treatment combinations were imposed for 120 d, after which the plants were harvested. In summary, 40 plants were subjected to four light treatments combined with two levels of available soil water (FC and WD). The light treatments are defined as follows: (i) plants continuously grown under low light (LL), (ii) plants initially grown under low light and then transferred to high light (LH), (iii) plants initially grown under high light and then transferred to low light (HL), and (iv) plants continuously grown under high light (HH). Obviously, for the sampled leaves from the LL and HH treatments, both bud formation and blade expansion occurred under LL and HH conditions, respectively; for the sampled leaves from the LH treatment, the leaf bud was formed under low light and the leaf blade expansion took place in high light, and the opposite is true for the leaves sampled from the HL treatment. The experiment was arranged in a completely randomized design, with five plants in individual pots per treatment combination as replicates. The experimental plot included one plant per container. It must be emphasized that, in the WD treatments, the sampled leaves expanded following the initiation of water shortage. The chemical composition, CC, MC, and gas exchange (see below) were examined in leaves approximately 30 d after full blade expansion.

The available water was calculated through the values of soil volumetric moisture at both field capacities (−0.010 MPa) and permanent wilting points (−1.5 MPa), following a soil moisture retention curve. Previously, the weights of all of the pots were standardized, using the same substrate amounts. Subsequently, the pot weights at both field capacities and 30% available water were gravimetrically established using a balance with a precision of 0.1 g. The moisture levels were controlled by monitoring the weights of the pots. Adjustments in pot weights due to increased biomass over time were carried out every 2 weeks.

Throughout the experiment, the plants were irrigated every 2 d and were fertilized with 1.5 g N, 0.6 g P, and 0.9 g K (per plant) and micronutrients at 90 d intervals. The average air temperature was 25.7 °C (maximum and minimum average temperatures of 27.2 °C and 16.8 °C, respectively), and the relative humidity was 77.4%, as measured by sensors installed at the weather station at the experimental site. The photosynthetically active radiation (PAR) was $19.0 \pm 1.6 \text{ mol m}^{-2} \text{ d}^{-1}$ in full sun and $2.9 \pm 0.3 \text{ mol}$

$\text{m}^{-2} \text{ d}^{-1}$ in the shade, measured using LI-190SA quantum sensors (Li-Cor) positioned 1 m above the plant canopies. All of the sensors were connected to an LI-1400 data logger (Li-Cor) that acquired data from the sensors every minute and stored them as 5 min averages.

Growth analysis

Destructive harvests were performed before applying the watering treatments and at the end of the experiment. At each harvest, plant tissues were oven-dried at 70 °C for 72 h, after which the total dry matter was determined. The relative growth rate (RGR) was estimated according to the method described by Hunt *et al.* (2002). The specific leaf area (SLA) was computed using the dry mass of 20 leaf discs (1.7 cm diameter each).

Chemical composition of leaf tissues

The chemical composition was analysed as described in Poorter and Villar (1997), with modifications. The leaves were collected, placed in liquid nitrogen, freeze-dried, ground sufficiently in a ball mill to pass through a 0.080 mm sieve, and then oven-dried at 60 °C for 48 h. All of the subsequent analyses were performed in duplicate.

A 10 mg sample was used to measure the C and N contents with an elemental analyser (Carlo Erba, Milan, Italy), as well as the relative abundances of ^{13}C and ^{12}C using a mass spectrometer (ANCA-GSL 20-20, Sercon, Crewe, UK) as described in Pinheiro *et al.* (2005). From these values, the carbon isotope composition ratio ($\delta^{13}\text{C}$) was estimated. The nitrate (NO_3^-) concentration was determined using a second sample (100 mg) based on the method reported by Cataldo *et al.* (1975). The concentration of organic N (N_{org}) was determined by subtracting the NO_3^- concentration from the total N. A third sample (200 mg) was used to estimate the ash content after the combustion of the sample in a muffle furnace at 550 °C for 12 h. The total amount of carbonates was estimated by quantitatively transferring the ash to an Erlenmeyer flask and determining ash alkalinity by first adding 0.05 N HCl and then titrating back with 0.05 N NaOH, using 0.1% methyl orange as pH indicator. The organic acid (OA) concentration was estimated by subtracting the NO_3^- content from the ash alkalinity and multiplying by an average molecular weight of 62.5. The concentration of minerals (MIN) was determined by multiplying the ash alkalinity (in meq g^{-1}) by 30 g eq^{-1} (mass of carbonate), subtracting this value from the total ash, and adding the weight of NO_3^- .

A fourth sample (250 mg) was used to determine the amount of a range of other compounds. Four millilitres of a mixture of methanol/chloroform (1:1, v/v) was added to each sample (Bligh and Dyer, 1959), stirred for 30 min, and then centrifuged at 4000 g for 5 min. The supernatant was collected, 2 ml of water was added while stirring, the mixture was centrifuged (4000 g, 5 min), and the chloroform phase was separated from the methanol/water phase. The lipid (LIP) concentration was determined gravimetrically after the evaporation of the chloroform phase in an oven at 60 °C. The concentration of total soluble phenols (PHE) was determined from the methanol/water phase with a colorimetric assay (at 725 nm) using the Folin–Ciocalteu reagent with tannic acid as a standard. The concentration of total soluble amino acids (AA) was determined according to DaMatta *et al.* (1999), and the concentration of total soluble sugars (TSS) was determined using the anthrone method with glucose as a standard (Fales, 1951). The pellet resulting from the chloroform–methanol–water extraction was boiled for 3 h at 100 °C with 3% HCl to break down the starch (STA), and the amount of sugar released from the acid hydrolysis were determined (Fales, 1951). Tubes containing known concentrations of starch were subjected to similar procedures to determine the STA content via a standard curve. The residue that remained after the acid hydrolysis, consisting of proteins (PRO) and cell wall compounds, was quantified gravimetrically after drying at 60 °C for 24 h. The cell wall content was determined

after subtracting the PRO content, which was determined by multiplying the N_{org} concentration present in the residue by 6.25 (Merino *et al.*, 1984). The PRO content in both the chloroform and methanol/water phases was checked using the Bradford method and was found to be negligible, that is, usually below 0.1% on the basis of dry weight.

The residue that remained after both the acid hydrolysis and drying at 60 °C was placed in a polyethylene vial, and 80 ml of a neutral detergent solution (Mertens, 2002) without sodium sulphite was then added in combination with a thermostable α -amylase (Termamyl 2X, Novozymes). The vials were sealed and autoclaved at 105 °C for 1 h. The insoluble material was retained by filtration under vacuum and was then sequentially washed with hot water and acetone. The contents of the fibres that were insoluble in neutral detergent (FND) were quantified after drying at 105 °C for 16 h. Subsequently, the contents of the fibres that were insoluble in acid detergent (FAD) were quantified after the addition of 80 ml of acid detergent (Van Soest and Robertson, 1985) followed by the same autoclaving, filtration, washing, and drying process described above. The concentration of hemicellulose (HCE) was obtained from the difference between the contents of the FND and the FAD. The residue was subjected to harsh acid hydrolysis, using 12 M H_2SO_4 at room temperature for 3 h. After vacuum filtration, washing with hot distilled water, and drying at 105 °C for 16 h, the concentration of cellulose (CEL) was estimated from the difference between the content of the FAD and the residue obtained after hydrolysis with H_2SO_4 . The residue that was insoluble in H_2SO_4 was quantified and subsequently combusted in a muffle furnace (500 °C, 3 h). The lignin (LIG) content was estimated as the loss of organic matter during incineration.

A fifth sample (50 mg) was used for determining the total methylxanthine alkaloids (ALK). After adding 2.5% (v/v) H_2SO_4 , the mixture was incubated at 90 °C for 60 min and then centrifuged at 4000 g for 5 min. The alkaloid content was determined from the supernatant by spectrophotometry at 274 nm based on the method reported by Khanchi *et al.* (2007), using caffeine as a standard.

Construction and maintenance costs of leaf tissues

Because of the variety of methodologies for estimating the CC and in order to allow useful comparisons of the present data with that previously reported by several authors (values for CC obtained by different methods for the same sample can vary by up to 20%: Williams *et al.*, 1987; Griffin, 1994), the CC were estimated using two of the predominantly employed methods: the method of Williams *et al.* (1987) and that of Vertregt and Penning de Vries (1987) modified by Poorter (1994), hereafter referred to as CC_W and CC_P , respectively. The CC_W was estimated using the following equation:

$$CC_W = \left([0.06968 \times Hc - 0.065] \times [1 - A] + [7.5 \times k \times N_{\text{org}} / 14.0067] \right) / Eg$$

where Hc is the heat of combustion (kJ g^{-1}) determined via the complete combustion of 500 mg of plant material in an adiabatic calorimeter bomb (Villar and Merino, 2001), A is the ash concentration (g g^{-1}), k is the oxidation state of the nitrogen source (+5 for nitrate or -3 for ammonium; as the N source was not unique, the mean value [+1] was used), N_{org} is the concentration of leaf organic N (g g^{-1}), and Eg is the leaf growth efficiency (0.89, according to Williams *et al.*, 1987).

The CC_P was estimated using the concentrations of C, MIN and N_{org} , according to the following equation:

$$CC_P = (-1.041 + 5.077 \times C) \times (1 - \text{MIN}) \times 5.235 \times N_{\text{org}}$$

The leaf costs of maintenance (MC) per unit dry mass ($\text{mg glucose g}^{-1} \text{DW d}^{-1}$) were determined following Penning de Vries

et al. (1974), using the following maintenance coefficients reported by Merino *et al.* (1984): 0.0425 (lipids), 0.0405 (protein), and 0.008 (ionic concentration).

CO_2 exchanges and payback time

The rates of photosynthesis and respiration were measured using a portable open-flow gas exchange system (Li-Cor 6400XT, Li-COR, Lincoln, Nebraska, USA). The mass-based net CO_2 assimilation rate (A_m) was derived from the SLA values and area-based momentary photosynthesis (estimated as a function of the PAR response curve obtained at 25 °C and 40 Pa CO_2 partial pressure). Further details have been given elsewhere (Cavatte *et al.*, 2011). Mass-based dark respiration (R_m) was estimated by integrating the measured night respiration rate obtained at three time points between sunset and sunrise. To compute the daily net C gain, a simple model was used (Poorter *et al.*, 2006). For this purpose, the daily rate of CO_2 fixation was estimated by taking the average time between sunrise and sunset during the period of imposition of the watering treatments (13 h photoperiod) and the frequency of different PAR intensities as measured above the canopies (PAR was measured at 5 min intervals in the two light treatments to compute a mean daily PAR curve). The daily carbon gain for the young, fully expanded leaves was estimated for each treatment using the A_m values integrated over the entire photoperiod ($\int A_m$) minus the night respiration integrated over the night period ($\int R_m$).

Estimates of payback times (PBT, expressed in days) were made assuming that all of the sugar fixed throughout the lifetime of a leaf has an equal value to the plant and that no leaf respiration is involved in growth processes, as was described by Poorter *et al.* (2006):

$$PBT = \frac{CC}{(\int A_m - \int R_m) \times 12 \times \frac{180}{172}}$$

where CC is the construction costs determined according to Williams *et al.* (1987), and the right side of the equation represents the calculation used to convert the moles of C into grams of glucose.

Statistical analysis

Mean (\pm SE) values were used to test the differences among the effects of the varied light ($n=4$) and water ($n=2$) treatments on each variable using a two-way ANOVA with type III sums of squares. Significant differences (at $P \leq 0.05$) among the light and water treatments were analysed using the Newman-Keuls *post hoc* test for multiple comparisons and the t test, respectively. To study the relationships among variables, the techniques of Pearson's linear correlation and principal component analysis (PCA) were employed using SAEG software, version 9.1 (SAEG, 2007).

Results

Most of the variables studied showed stronger responses to changes in light availability than to the water factor (Table 1). Overall, the traits showed weak or negligible responses to the light \times water interaction (Table 1).

Regardless of the water availability, the RGR was higher in the LH/HH than in the LL/HL plants (Fig. 1a), whereas an opposite response was found for the SLA (Fig. 1b). Independent of the water supply, the SLA was, on average, 19% higher in LL than in HL leaves and 8% higher in LH than in HH leaves (Fig. 1b).

Table 1. Percentage of the total sum of squares in the ANOVA that were explained by the effect of light, water and the light×-water interaction, as well as the significance values

CV is the coefficient of variation (%). See details for other abbreviations in the Abbreviations section. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and ^{ns} $P > 0.05$.

Traits	Water	Light	Water×light	CV
RGR	13***	56***	7*	17.1
SLA	2 ^{ns}	64***	8*	5.9
A_m	31***	53***	14***	9.2
R_m	8***	80***	3 ^{ns}	8.9
$\delta^{13}C$	5**	61***	18***	3.1
LIP	13**	20*	12 ^{ns}	6.4
PRO	9 ^{ns}	28 ^{ns}	1 ^{ns}	16.1
PHE	< 1 ^{ns}	88***	1 ^{ns}	17.7
LIG	< 1 ^{ns}	45**	2 ^{ns}	20.7
CEL	4*	62***	12**	14.9
HCE	< 1 ^{ns}	72***	7*	8.4
ALK	2*	89***	5***	5.7
STA	65***	10**	9**	17.4
TSS	13***	57***	16***	23.2
MIN	< 1 ^{ns}	68***	11**	14.1
OA	16***	46***	6 ^{ns}	12.0
AA	< 1 ^{ns}	69***	3 ^{ns}	13.6
N	21***	35***	22***	5.1
C	< 1 ^{ns}	82***	6**	1.1
H_c	1 ^{ns}	74***	1 ^{ns}	2.8
CC_w	1 ^{ns}	79***	2 ^{ns}	3.1
CC_p	4**	78***	9***	1.7
MC	19***	37***	3 ^{ns}	2.8
PBT	62***	30***	<1 ^{ns}	13.5

Both the A_m and R_m were, on average, higher in LH/HH than in LL/HL leaves (96% and 46%, on average, respectively; Fig. 1c, d), although HH leaves displayed significantly higher A_m and R_m than their LH counterparts. A_m was lower under WD conditions (44% on average), particularly in LH/HH leaves; notably, WD led to increases in R_m (~31%) but only in LL/HL individuals (Fig. 1c, d). The $\delta^{13}C$ decreased in LL plants, with the opposite response in the plants from the other light treatments. The $\delta^{13}C$ was lower in LL/HL than in LH/HH leaves, with values of 1.66‰ and 3.66‰ when leaf expansion occurred under FC and WD conditions, respectively (Fig. 1e). Regardless, both the $\delta^{13}C$ and A_m were positively correlated with the RGR (Table 2).

Small, if any, differences for the LIP and PRO pools among all the treatments were noted and, on average, the concentrations of LIP and PRO were approximately 8% and 6% of the leaf dry weight, respectively (Fig. 2a, b). The light and water factors explained only a small proportion of the total variation of the concentrations of these compounds (Table 1). Light was the main factor responsible for the differences in the leaf chemical composition associated with the other constituents analysed (as assessed by percentage of dry weight), with the exception of STA, which was approximately 8% in the leaves expanded under FC and 4% in the leaves expanded under WD (Fig. 2). The

LL/HL and LH/HH leaves, respectively, were composed of 3% and 8% PHE; 5% and 6% LIG; 13% and 8% CEL; 15% and 10% HCE; 2% and 4% ALK; 0.3% and 0.5% AA; 2% and 5% TSS; 6% and 3% MIN, and 8% and 6% OA (Fig. 2). Changes in the water availability contributed to the variations in TSS, OA, and MIN. On average, both TSS and MIN were larger (approximately 25%) and OA was lower (16%) in the FC-expanded leaves when compared with their WD counterparts (Fig. 2i–k).

The light environment of mature leaves had a minimal effect on the leaf chemical composition of the foliage expanded in the new light environment (Fig. 2), although there were two consistent exceptions: PHE, which were ~23% lower in LH than in HH leaves (Fig. 2c) and AA, which were 26% lower in HL than in LL leaves (Fig. 2l).

The values of the CC_w ranged from 1.176 g glucose g⁻¹ DW to 1.380 g glucose g⁻¹ DW; overall, these values were 10% lower than those of the CC_p (Fig. 3a, b). On average, the LH/HH leaves had 11–12% larger CC_w and CC_p than the LL/HL ones (Fig. 3a, b). However, the CC_w and CC_p were well correlated to each other ($r=0.91$; Table 2).

Insights into the chemical composition of the leaves are required to understand the differences in the CC between shade and sun leaves, as the H_c and C concentrations are highly dependent on chemical composition. There were strong and positive correlations of both the H_c and C with LIP, PHE, TSS, AA, and ALK (Table 2). It must be emphasized that, among the chemical constituents, PHE and ALK showed the strongest correlations with the CC_w (PHE, $r=0.83$; ALK, $r=0.71$) and the CC_p (PHE, $r=0.89$; ALK, $r=0.85$). Overall, there were negative correlations between compounds with high energy costs, represented by the values of PHE and ALK and low energy costs, represented by the values of CEL, HCE, OA, and MIN (Table 2). Increasing the PHE and ALK concentrations was positively correlated with the A_m , $\delta^{13}C$, and TSS, although there was no significant correlation with the STA. It should also be noted that the CC_w , CC_p , PHE, and ALK were all negatively correlated with the SLA but positively correlated with the RGR (Table 2).

A trend towards larger (5%) MC was found under WD conditions, reaching statistical significance in LL and HH leaves. Regardless of the water supply, the MC did not differ among LL, HL, and HH leaves; on average, these leaves had a 6% lower MC than that of the LH leaves (Fig. 3c). Variations in the MC were chiefly correlated with the LIP ($r=0.57$), PRO ($r=0.77$), and AA ($r=0.67$) (Table 2). No significant correlation between the MC and RGR was observed, whereas, unexpectedly, the MC were negatively correlated with the SLA ($r=-0.41$) (Table 2).

The PBT ranged from 13.1 d to 66.9 d and was dramatically lower in the LH/HH than in the LL/HL leaves (Fig. 3d). However, the PBT was 40% greater in LH leaves than in their HH counterparts, which could be a reflection of the lower A_m of the LH leaves. There was no interactive effect between the water and light supplies on the PBT. However, as expected, the WD contributed greatly to the PBT, which increased up to 169% in the case of the HH

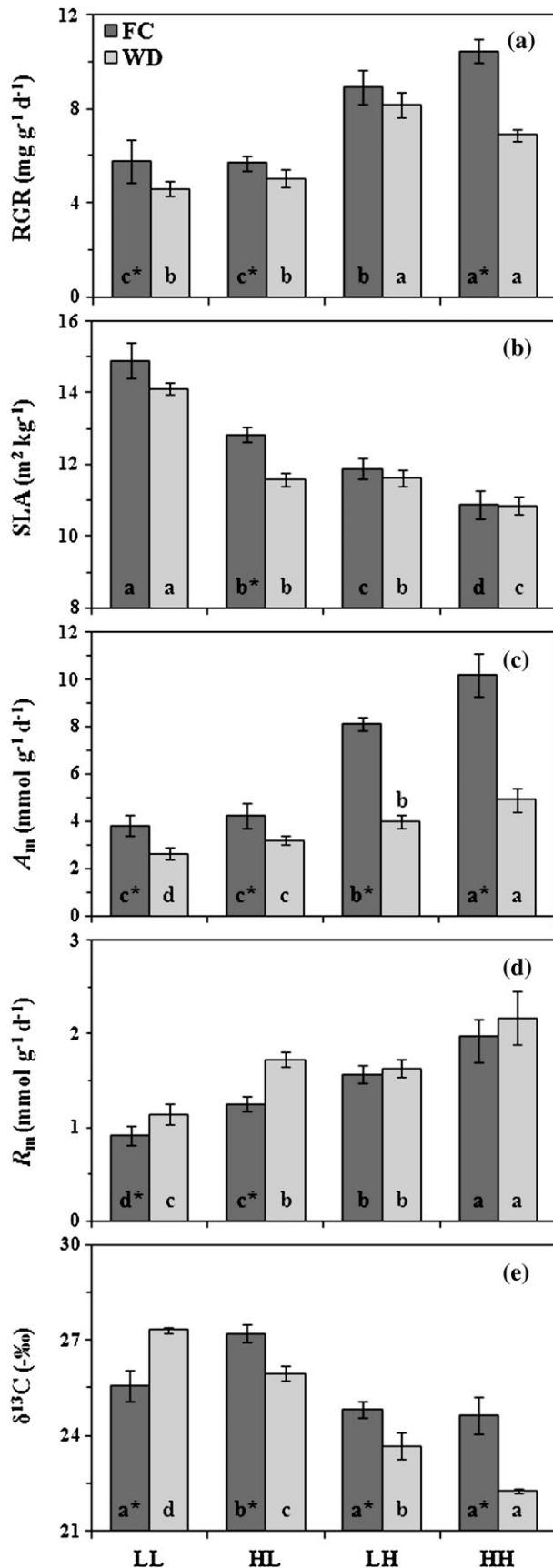


Fig. 1. Means for the relative growth rate (RGR), specific leaf area (SLA), mass-based net carbon assimilation rate integrated over the diurnal period (A_m), mass-based dark respiration rate integrated

leaves (Fig. 3d). The PBT correlated significantly with the A_m ($r = -0.83$), RGR ($r = -0.67$), and $\delta^{13}\text{C}$ ($r = -0.51$), but not with R_m ($r = -0.18$) (Table 2). The larger PBT of the LL/HL leaves could, to a certain extent, be compensated for by the longer lifespan of leaves under low rather than in high light. In fact, leaf abscission occurred at 394 ± 14 d after the full blade expansion in HH plants; in LL plants, the tagged leaves were 420-d old after full expansion when the last destructive harvest was performed, without any obvious signs of senescence (data not shown).

Considering that the largest proportion of the variation in the CC was explained by the light factor and that approximately 83% of the total dry mass was recovered in the chemical composition analysis (data not shown), an examination of the contribution of each component in the variation of the CC was made only for the light factor, based on the method reported by Poorter and DeJong (1999) (Fig. 4). Regardless of the water availability, approximately 95% of the differences in the CC in the leaves submitted to varying light availability were explained by the changes in PHE (49%), ALK (23%), MIN (13%), and LIG (10%). The concentration of the TSS changed greatly depending on the light treatments, but did not contribute significantly or to an extent to which it could explain the differences in the CC (Table 2, Fig. 4).

Two components of the PCA explained 79% of the total data variation. The following four different groups of variables were observed (Fig. 5): (i) variables positively correlated with the first component: RGR, TSS, PHE, ALK, $\delta^{13}\text{C}$, A_m , R_m , CC_w , and CC_p ; (ii) variables negatively correlated with the first component: SLA, CEL, HCE, OA, MIN, and PBT; (iii) variables positively correlated with the second component: MC, PRO, and LIP; and (iv) variables negatively correlated with the second component: STA. LIG did not correlate significantly with any principal component.

Discussion

This study compares, for the first time, the combined effects of light and water availability on the functional relationships among RGR, the leaf chemical composition, the CC

over the night period (R_m) and carbon isotope composition ratio ($\delta^{13}\text{C}$) of coffee plants subjected to four light treatments (LL, plants continuously grown under low light; HL, plants initially grown under high light and then transferred to low light; LH, plants initially grown under low light and then transferred to high light; HH, plants continuously grown under high light) combined with two water [field capacity (FC, 100% of available water) and water deficit (WD, 30% of available water)] supplies. Within each water supply, the means followed by the same letter did not differ significantly (Newman-Keuls *post hoc* test) among light treatments; when shown, asterisks indicate significant differences (*t* test) between water supplies within the same light treatment. $n = 5 \pm \text{SE}$, $P \leq 0.05$.

Table 2. Pearson product moment correlations between the average chemical composition [lipids, LIP; lignin, LIG; total soluble phenols, PHE; protein, PRO; cellulose, CEL; hemicellulose, HCE; starch, STA; total soluble sugars, TSS; organic acids, OA; minerals, MIN; amino acids, AA; total methylxanthine alkaloids, ALK; nitrogen, N; carbon, C; heat of combustion, H_c ; leaf construction costs determined according to Williams *et al.* (1987), CC_W ; leaf construction costs determined according to Poorter (1994), CC_P ; total maintenance costs, MC; mass-based net carbon assimilation rate, A_m ; mass-based respiration rate, R_m ; carbon isotope composition ratio, $\delta^{13}C$; payback times, PBT; specific leaf area, SLA; and relative growth rate, RGR] of coffee plants grown under contrasting light and water conditions; $n=40$; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and $^{ns}P > 0.05$.

	LIG	PHE	PRO	CEL	HCE	STA	TSS	OA	MIN	AA	ALK	N	C	H_c	CC_W	CC_P	MC	A_m	R_m	$\delta^{13}C$	PBT	SLA	RGR
LIP	-0.12 ^{ns}	0.16 ^{ns}	0.10 ^{ns}	-0.09 ^{ns}	-0.24 ^{ns}	-0.30*	0.17 ^{ns}	0.08 ^{ns}	-0.33*	0.46**	0.25 ^{ns}	0.12 ^{ns}	0.16 ^{ns}	0.32*	0.35*	0.23 ^{ns}	0.57***	-0.26 ^{ns}	0.04 ^{ns}	0.35*	0.17 ^{ns}	0.13 ^{ns}	0.06 ^{ns}
LIG		0.19 ^{ns}	-0.15 ^{ns}	-0.48***	0.20 ^{ns}	-0.17 ^{ns}	0.04 ^{ns}	-0.24 ^{ns}	0.14 ^{ns}	-0.06 ^{ns}	0.20 ^{ns}	0.17 ^{ns}	0.18 ^{ns}	0.11 ^{ns}	0.09 ^{ns}	0.27*	0.20 ^{ns}	0.07 ^{ns}	0.27*	0.27*	-0.03 ^{ns}	-0.30*	0.12 ^{ns}
PHE			0.15 ^{ns}	-0.76***	-0.92***	-0.10 ^{ns}	0.62***	-0.84***	-0.77***	0.61***	0.88***	0.32*	0.82***	0.73***	0.83***	0.89***	0.25 ^{ns}	0.64***	0.80***	0.72***	-0.31*	-0.79***	0.61***
PRO				-0.21 ^{ns}	-0.24 ^{ns}	-0.55***	-0.12 ^{ns}	0.01 ^{ns}	-0.03 ^{ns}	0.50***	0.04 ^{ns}	0.56***	0.13 ^{ns}	0.40**	0.35*	0.40*	0.77***	-0.22 ^{ns}	0.02 ^{ns}	0.10 ^{ns}	0.15 ^{ns}	-0.35*	0.09 ^{ns}
CEL					0.73***	0.07 ^{ns}	-0.60***	0.56***	0.65***	-0.47**	-0.78***	-0.15 ^{ns}	-0.76***	-0.71***	-0.73***	-0.75***	-0.34*	-0.37**	-0.67***	-0.71***	0.36*	0.59***	-0.51***
HCE						-0.06 ^{ns}	-0.76***	0.74***	0.79***	-0.63***	-0.70***	-0.17 ^{ns}	-0.66***	-0.79***	-0.86***	-0.81***	-0.37*	-0.56***	-0.55***	-0.54***	0.45**	0.74***	-0.55***
STA							0.40*	-0.23 ^{ns}	-0.14 ^{ns}	-0.25 ^{ns}	-0.07 ^{ns}	-0.49**	0.11 ^{ns}	0.08 ^{ns}	0.08 ^{ns}	-0.11 ^{ns}	-0.55***	0.67***	-0.12 ^{ns}	-0.12 ^{ns}	-0.74***	0.11 ^{ns}	0.38**
TSS								-0.46**	-0.74***	0.19 ^{ns}	0.50***	-0.43**	0.56***	0.67***	0.69***	0.46**	0.17 ^{ns}	0.75***	0.48***	0.45**	-0.72***	-0.44**	0.56***
OA									0.59***	-0.42**	-0.72***	-0.40**	-0.63***	-0.52***	-0.65***	-0.74***	0.05 ^{ns}	-0.61***	-0.43**	-0.40*	0.49***	0.73***	-0.60***
MIN										-0.28*	-0.58***	0.10 ^{ns}	-0.66***	-0.63***	-0.74***	-0.64***	-0.02 ^{ns}	-0.44**	-0.54***	-0.70***	0.36*	0.44**	-0.52***
AA											0.67***	0.60***	0.58***	0.67***	0.68***	0.74***	0.67***	0.34*	0.24 ^{ns}	0.55***	0.18 ^{ns}	-0.61***	0.44**
ALK												0.28*	0.81***	0.61***	0.71***	0.85***	0.22 ^{ns}	0.51***	0.76***	0.79***	-0.22 ^{ns}	-0.82***	0.60***
N													-0.01 ^{ns}	0.19 ^{ns}	0.24 ^{ns}	0.53***	0.35*	-0.50***	-0.01 ^{ns}	0.06 ^{ns}	0.41**	-0.54***	-0.42**
C														0.80***	0.83***	0.97***	0.21 ^{ns}	0.62***	0.76***	0.72***	-0.46**	-0.60***	0.69***
H_c															0.98***	0.83***	0.54***	0.58***	0.52***	0.58***	-0.44**	-0.65***	0.69***
CC_W																0.91***	0.45**	0.47***	0.55***	0.64***	-0.45**	-0.70***	0.67***
CC_P																	0.45**	0.58***	0.75***	0.75***	-0.27*	-0.82***	0.44**
MC																		0.31*	0.08 ^{ns}	0.26 ^{ns}	0.15 ^{ns}	-0.41*	0.07 ^{ns}
A_m																			0.51***	0.31*	-0.83***	-0.43**	0.76***
R_m																				0.63***	-0.18 ^{ns}	-0.77***	0.44**
$\delta^{13}C$																					-0.28*	-0.29*	0.46**
PBT																						0.23 ^{ns}	-0.67***
SLA																							-0.27*

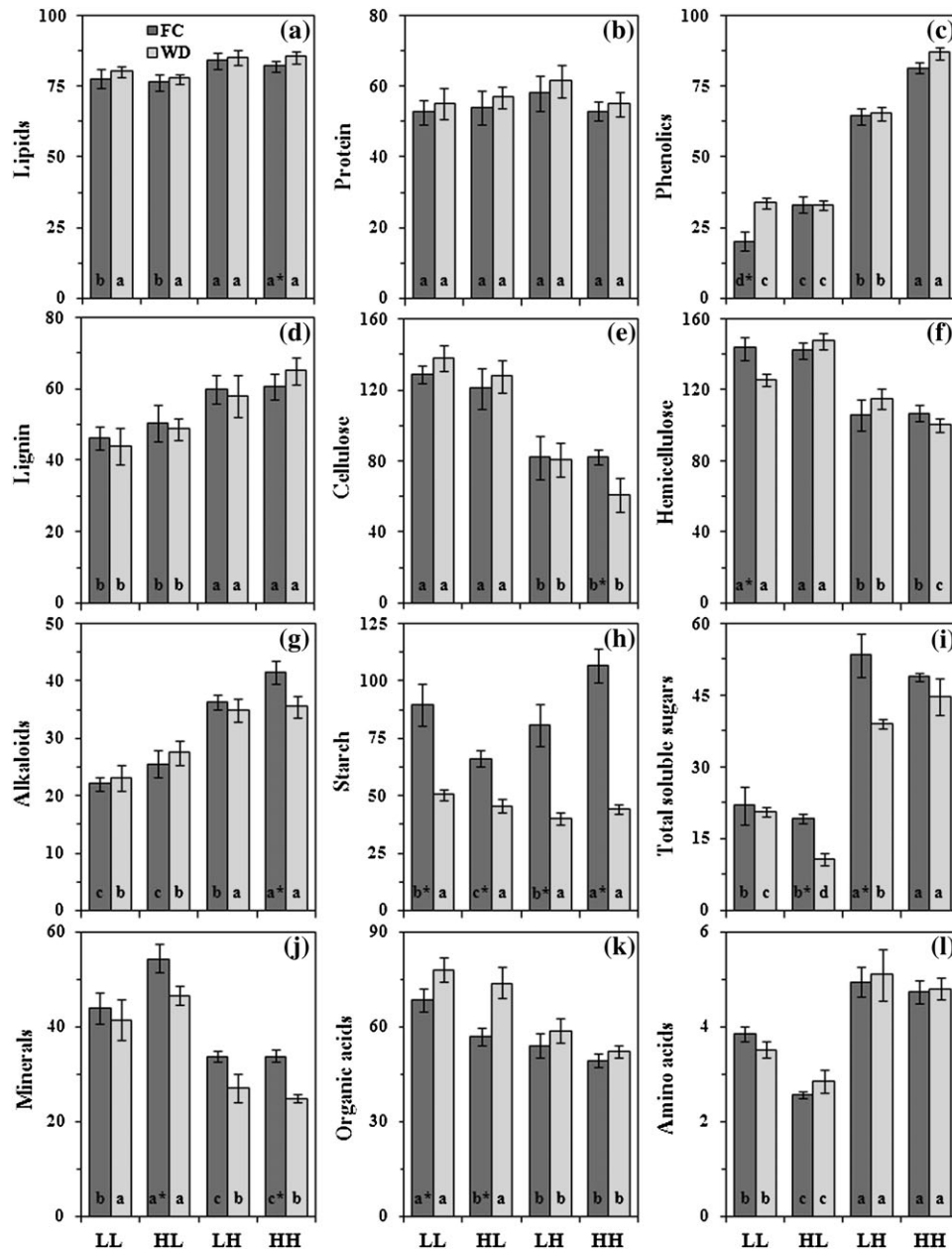


Fig. 2. Chemical composition in leaves of coffee plants subjected to four light treatments (LL, plants continuously grown under low light; HL, plants initially grown under high light and then transferred to low light; LH, plants initially grown under low light and then transferred to high light; HH, plants continuously grown under high light) combined with two water [field capacity (FC, 100% of available water) and water deficit (WD, 30% of available water)] supplies. Concentrations are expressed in mg g^{-1} DW. Statistics as in Fig. 1.

and MC, and the benefits in terms of the PBT. It was found that the RGR was positively associated with the PHE and ALK pools, which are compounds related to survival (Agrawal and Fishbein, 2006). These compounds, in turn, were negatively correlated with the SLA, suggesting that they might be associated with greater protection against herbivory. Therefore, the relationships between the chemicals and the RGR found in the present study do not support our working hypothesis, that is, there are no apparent trade-offs with regard to the potential negative effects on plant growth and fitness due to the diversion of resources to secondary metabolites associated with defence.

Recent results obtained with loblolly pine clones by Aspinwall *et al.* (2011), who investigated genetic and growth-related effects on foliar concentrations of total PHE, also do not support the existence of a trade-off between growth and defence.

In addition to contributing to plant defences against biotic stresses (such as ALK), higher PHE concentrations may also significantly contribute to protection against the excessive energy (Close *et al.*, 2003; Wilhelm and Selmar, 2011). Several studies have shown that coffee plants, despite displaying low net photosynthetic rates (typically ranging from 4–10 $\mu\text{mol m}^{-2} \text{s}^{-1}$) that are saturated at relatively low

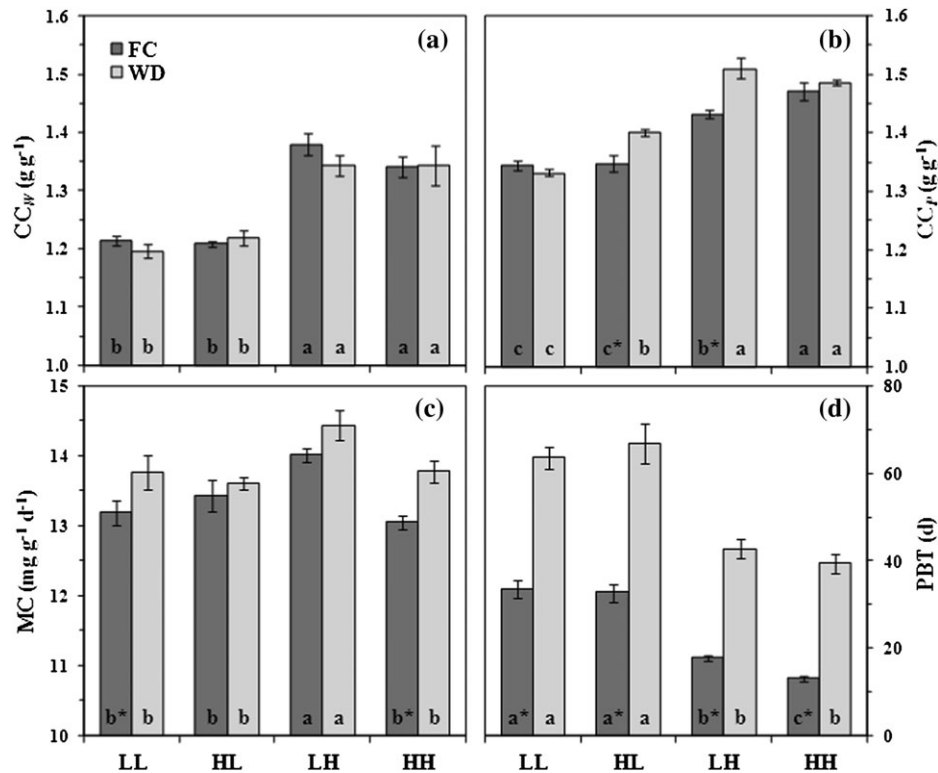


Fig. 3. Means for the leaf construction costs determined according to Williams *et al.* (1987) (CC_w) and Poorter (1994) (CC_p), total maintenance costs (MC) and payback times (PBT) in leaves of coffee plants subjected to four light treatments (LL, plants continuously grown under low light; HL, plants initially grown under high light and then transferred to low light; LH, plants initially grown under low light and then transferred to high light; HH, plants continuously grown under high light) combined with two water [field capacity (FC, 100% of available water) and water deficit (WD, 30% of available water)] supplies. Statistics as in Fig. 1.

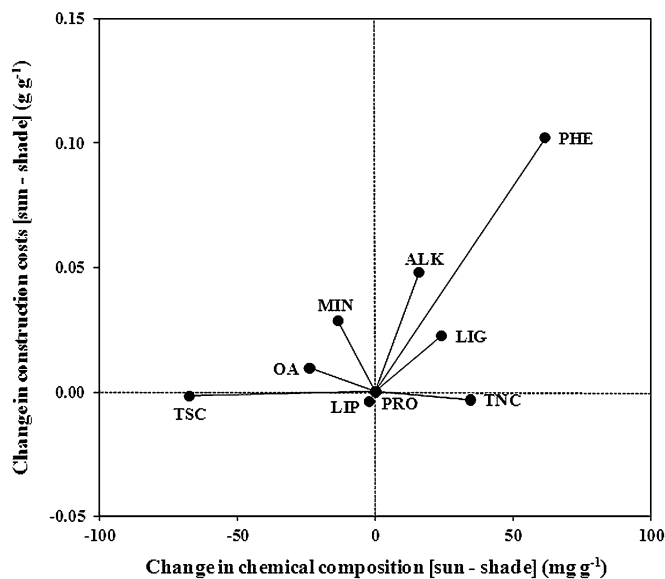


Fig. 4. Changes in leaf construction costs, determined according to Williams *et al.* (1987), as a function of chemical compounds (alkaloids, ALK; organic acids, OA; total structural carbohydrates, TSC; non-structural carbohydrates, TNC; total soluble phenols, PHE; lignin, LIG; lipids, LIP; proteins, PRO; minerals, MIN) observed for high- and low-light leaves of coffee plants.

PAR (DaMatta, 2004), are well protected against photo-inhibitory damage in open fields (Chaves *et al.*, 2008; Moraes *et al.*, 2010; Pompelli *et al.*, 2010). Therefore, it is proposed that the high PHE concentration, as noted in the LH/HH plants, may have an important role in protecting the coffee leaves against photoinhibition, as has already been proposed for *Eucalyptus nitens* grown under limiting light and nutrient conditions (Close *et al.*, 2003). Furthermore, the diversion of C that is in relative excess to the expensive synthesis of ALK and PHE may act as an additional mechanism to dissipate the excess energy and to rechannel photoassimilates (especially under high light), which may help to avoid photosynthetic down-regulation and photoinhibition. Collectively, these data suggest that the capacity of the plant to increase its defence ability without compromising growth may be an acclimative strategy that allows the coffee tree to grow and produce successfully under full sunlight conditions.

The carbon/nutrient balance hypothesis (Bryant *et al.*, 1983; Herms and Mattson, 1992) has been used to explain the effects of light (and nutrient) availability on the concentrations of both C- and N-based plant secondary metabolites, such as PHE and ALK. This hypothesis posits that the allocation of resources to secondary metabolism increases when resources are in excess of the growth requirements or when there is an imbalance in resource

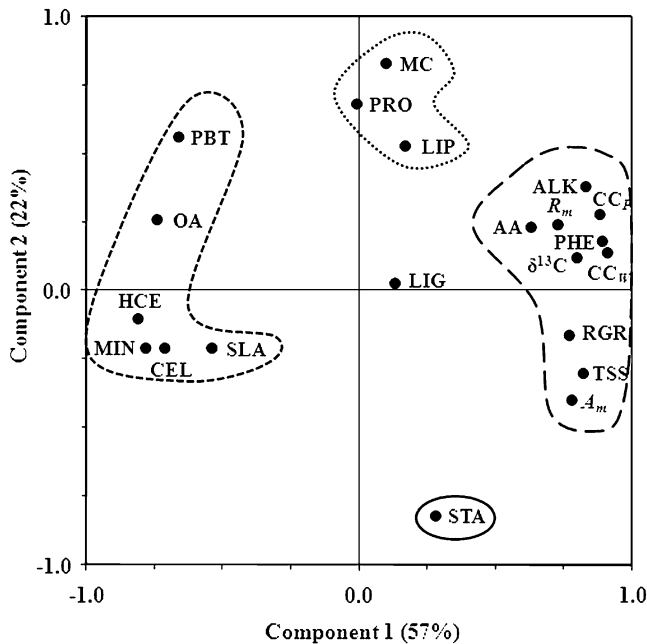


Fig. 5. Principal component analysis for chemical constituents (organic acids, OA; total soluble sugars, TSS; starch, STA; amino acids, AA; cellulose, CEL; total soluble phenols, PHE; hemicellulose, HCE; lignin, LIG; lipids, LIP; proteins, PRO; minerals, MIN; alkaloids, ALK), mass-based net carbon assimilation rate integrated over the diurnal period, A_m ; mass-based dark respiration rate integrated over the night period, R_m ; carbon isotope composition ratio ($\delta^{13}\text{C}$), leaf construction costs determined according to Williams *et al.* (1987) (CC_W) and Poorter (1994) (CC_P), total maintenance costs (MC), specific leaf area (SLA), payback times (PBT), and relative growth rate (RGR) of coffee plants grown under contrasting light and water conditions.

supply. It is shown here that PHE and ALK were both positively correlated with the A_m and carbohydrate pools, suggesting that C that is in relative excess could be diverted to secondary metabolism. Furthermore, because the leaf N concentration was quite similar among the treatments (data not shown), N availability could not be invoked to explain the differences in the ALK (and PHE) concentration found. Thus, the larger ALK and PHE pools given an ample resource supply are clearly consistent with the carbon/nutrient balance hypothesis.

The leaf CC were remarkably highly associated with the levels of the ALK and PHE pools, and were 12% larger for the LH/HH plants than for the LL/HL ones. Such a difference is larger than that (5% at most) reported by Baruch *et al.* (2000) who evaluated individual plants from other woody tropical species grown under greenhouse conditions in low and high light. In contrast to many studies covering a number of different species (Poorter *et al.*, 2006; Villar *et al.*, 2006), where positive correlations between compounds of high and low energy cost have been found, which could buffer variations in the CC, a negative correlation was found between both PHE and ALK (expensive classes of compounds) with CEL, HCE, OA,

and MIN (compounds of low energy cost); also no significant correlation was found between PHE and ALK with other compounds of high energy cost (LIP, PRO, and LIG). This finding may explain the large differences between the CC when comparing the LH/HH leaves with their LL/HL counterparts.

The differences in the MC between the LL/HL and LH/HH leaves were relatively narrow, with no significant impact on the RGR. Conversely, the LH/HH leaves had higher CC in comparison with their shade counterparts, but due to their shorter lifespan, the sun leaves would have a shorter useful period in terms of C fixation than the LL/HL leaves (Villar and Merino, 2001). As the PCA suggests, the CC were directly associated with both the RGR and A_m , and, therefore, the leaves with higher CC had a greater C gain, such that the costs necessary for the construction of the leaf tissue would be quickly amortized, that is, they had a lower PBT, as was found for the LH/HH leaves. This finding suggests that the leaf lifespan of coffee plants is more closely related to the C gain per se than to the CC.

The light environment of the mature leaves (which were fully developed before the reciprocal transfers of the plants between the light environments) could, to some extent, affect the SLA and, presumably, the anatomy of the leaves expanded upon transfer (Matos *et al.*, 2009). In addition, and despite the minor (if any) alterations in both the CC and leaf chemical composition when comparing the HL and LL leaves, and the LH with their HH counterparts, compelling evidence was found that the physiology of the leaves that expanded upon transfer was also altered. This was demonstrated, for example, by the lower A_m and R_m in LH leaves compared with HH leaves, despite the larger SLA in the former. Similar results to those found for A_m were also reported in the *Fagus* species (Uemura *et al.*, 2000). Moreover, the LH leaves displayed the largest MC of the other leaf groups, which might suggest higher costs for these leaves to acclimate to the new, potentially more stressful, environment upon the transfer from low to high light. Taking this information together, it might be proposed that an anticipated irradiance signal originating in mature leaves (Lake *et al.*, 2001; Jiang *et al.*, 2011) may, to some extent, affect not only the morphology/anatomy but also the physiology of the newly expanding coffee leaves.

Conclusions

The light factor greatly influenced the leaf chemical composition and, thus, the CC, whereas the water factor, by constraining carbon fixation, dramatically affected the PBT. It is proposed that an increased RGR in coffee growing under varying water and light supplies could be obtained in plants with leaves under the following conditions: (i) higher CC due to the higher PHE and ALK concentrations, which are related to defence against stresses; (ii) lower concentrations of CEL and HCE, which are associated with a lower SLA and, consequently, higher photosynthetic rates per unit area and water-use efficiency

(Aranda *et al.*, 2007), as also judged from the negative correlation between the SLA and $\delta^{13}\text{C}$; and (iii) a lower PBT, contributing to a favourable C balance, namely, higher concentrations of carbohydrates as a way to defray possible costs in an attempt to overcome periods of resource limitation. In summary, the sun-grown coffee plants fixed more C for growth and secondary metabolism, with the net effect of an increased RGR.

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