NELSON FACUNDO RODRÍGUEZ-LÓPEZ

ECOPHYSIOLOGICAL ACCLIMATION OF COFFEE (*Coffea arabica* AND *C. canephora*) PLANTS TO COPE WITH TEMPORAL FLUCTUATIONS OF LIGHT SUPPLY

Thesis submitted to Federal University of Viçosa, as part of the requirements for obtaining of *Doctor Scientiae* degree in Plant Physiology.

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ATTESTED IN: July 21th, 2012.

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To my mother Elvia and my father Efrain and my brothers Fabiola and Edgar,
for all their love, sacrifice and constant support, always present in my heart

With all my love to my wife Yenis, and my three treasures:
Esteban, Daniela and Sofia

To my mother in law Juana,
for her help and support throughout this adventure

In Memoriam

My friend and professor, Ernesto Arrieta Maza,
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BIOGRAPHY

NELSON FACUNDO RODRÍGUEZ-LÓPEZ was born in Cereté-Córdoba, Colombia on April 28th, 1971. In 1996, he graduated with a degree in Biology and Chemistry at the University of Córdoba, Monteria-Córdoba. In July of 2001, he obtained his *Master Scientiae* degree in Plant Physiology from the Federal University of Viçosa, Viçosa-MG, Brazil. In July 2003, he became an adjunct professor at the Industrial University of Santander, Bucaramanga-Santander. In July 2007, he began his doctoral studies in the Plant Physiology Program at the Federal University of Viçosa, Viçosa-MG, Brazil.

CONTENT

RESUMO

RODRÍGUEZ-LÓPEZ, Nelson Facundo, D.Sc., Universidade Federal de Viçosa, julho de 2012. **Aclimatação ecofisiológica do cafeeiro (*Coffea arabica* e *C. canephora*) para enfrentar às flutuações temporais da disponibilidade de luz**. Orientador: Fábio Murilo DaMatta. Coorientadores: Fernando Valladares Ros, Raimundo Santos Barros e Marília C. Ventrella.

Geralmente, assume-se que a quantidade total de luz disponível é o condutor principal para o crescimento e produtividade das plantas. Porém, como pouco se conhece acerca dos efeitos das variações temporais diurnas na disponibilidade da luz, no acúmulo de biomassa e no desempenho fisiológico, neste estudo se examinaram caracteres morfológicos, fisiológicos e bioquímicos em plantas de cafeeiro em dois experimentos independentes. No primeiro experimento, plantas de café arábica (*C. arabica*) foram submetidas a vários tratamentos de luz: totalmente sob 100%, 40% ou 10% de luz solar (S-100, S-40 e S-10, respectivamente), sob 40% ou 10% de luz solar durante a manhã e, em seguida, submetidas a 100% de luz solar até o anoitecer (S-40/100 e S-10/100, respectivamente), e em 100% de luz solar do amanhecer ao meio-dia e depois submetidas a 40% ou 10% de luz (S-100/40 e S-100/10, respectivamente). As plantas em S-100 acumularam maior biomassa, com alterações mínimas nas razões alométricas, quando comparadas com indivíduos de outros tratamentos. Essa maior biomassa foi, aparentemente, independente das taxas fotossintéticas por unidade de área foliar e de alterações na disponibilidade de carboidratos; sendo associado principalmente à rápida formação de área foliar com o aumento da disponibilidade de irradiância. Em contraste, o menor acúmulo de biomassa nas plantas em S-10/100 e S-10 deve ter sido conseqüência das limitações de carbono e de uma menor área foliar. O acúmulo de biomassa não foi dependente apenas da irradiância total interceptada, mas também das variações temporais do fornecimento de luz, como evidenciado pela comparação da maior biomassa (35%) em indivíduos em S-100/10 do que em S-10/100. As alterações nas taxas fotossintéticas entre os tratamentos foram aparentemente não relacionadas com o acúmulo de carboidratos ou fotoinibição. Em geral, em nível de folha, apenas pequenas alterações nos caracteres fisiológicos respondentes à luz foram observadas, principalmente, quando comparado os indivíduos em S-10 com aqueles dos outros tratamentos. É pouco provável que o maior acúmulo de biomassa e melhor desempenho fisiológico das plantas em S-100 sejam diretamente associados a ganhos de carbono por

unidade de área foliar, mas sim a processos morfogenéticos induzidos pela luz relacionados a uma rápida formação de área foliar, que, por sua vez, devem ter levado a uma maior produtividade fotossintética em nível de planta inteira. O acúmulo de biomassa em grande parte depende não unicamente da quantidade total de luz interceptada, mas também das escalas temporais de variações diurnas no suprimento de luz, isto é, a quantidade de luz recebida pelas plantas durante a manhã teve um papel importante para incrementar a biomassa. Esta informação tem importância prática para o uso de árvores de sombra em fazendas localizadas em zonas montanhosas, onde o uso do sombreamento deve ser evitado em terrenos com exposição para o oeste se um melhor crescimento (e produção) é o principal objetivo a ser alcançado. No segundo experimento, foi usado um desenho experimental onde dois clones de café robusta (*C. canephora*) foram consorciados com árvores de seringueira de modo que foi permitido a comparação de arbustos de café sombreados na manhã (SM) com aqueles sombreados na tarde (SA), e então comparando ambos com arbustos expostos a pleno sol durante o curso do dia (FS). Os arbustos em SM apresentaram melhor desempenho nas trocas gasosas ao longo do dia em comparação com aqueles em SA e FS, os quais exibiram limitações devido a fatores estomáticos (arbustos em SA) e bioquímicos (arbustos em FS). Os caracteres fisiológicos associados com a captura de luz mostraram uma maior resposta às variações temporais da luz do que à quantidade de luz recebida, embora esse comportamento possa ser uma resposta específica dos clones estudados. As atividades de enzimas do sistema antioxidativo exibiram diferenças mínimas quando comparando os clones em SM e SA, e, em contraste, foram maiores nos clones em FS. Independentemente dos tratamentos de luz, não foram encontrado sinais de fotoinibição ou danos celulares. A aclimatação à variação temporal da luz não teve custos adicionais aparentes para a construção e manutenção das folhas entre os tratamentos. Ambos os indivíduos em SM e SA apresentaram um alto retorno em termos de fluxo de investimento (maiores A_{max} em base de área e massa, PNUE e WUE no longo prazo, por exemplo) do que sua contraparte FS. Em resumo, o sombreamento na manhã pode melhorar o desempenho fisiológico do cafeeiro em ambientes marginais tropicais, no entanto, é importante selecionar genótipos com adequada plasticidade fenotípicos, como encontrada no clone 03, para lidar com a redução na disponibilidade de luz.

ABSTRACT

RODRÍGUEZ-LÓPEZ, Nelson Facundo, D.Sc., Universidade Federal de Viçosa, July, 2012. **Ecophysiological acclimation of coffee (*Coffea arabica* and *C. canephora*) plants to cope with temporal fluctuations of light supply**. Adviser: Fábio Murilo Da Matta. Co-advisers: Fernando Valladares Ros, Raimundo Santos Barros and Marília C. Ventrella.

It is generally assumed that the daily quantum input is what drives plant growth and productivity. However, as little is known about how temporal diurnal variations of light availability influence biomass accumulation and physiological performance, this study examined the morphological, physiological and biochemical traits of coffee plants in two independent experiments. In the first experiment, Arabica coffee (*C. arabica*) plants were grown in pots and subjected to seven light treatments as follows: plants grown entirely under 100%, 40% or 10% sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). The S-100 plants accumulated more biomass compared to plants grown under other treatments and showed minimal changes in biomass allocation. An increased biomass was associated with faster leaf area formation and increasing irradiance that was independent of the photosynthetic rates per unit leaf area and consequent changes in carbohydrate availability. In contrast, the lower biomass in S-10/100 and in S-10 individuals was likely a consequence of carbon limitations as well as decreased leaf areas. Changes in the photosynthetic rates between treatments were apparently unrelated to carbohydrate accumulation or photoinhibition. Overall, only minor physiological alterations in traits were observed at the leaf level; significant changes were only apparent in S-10 individuals with the other treatments. In summary, the growth and physiological performance of coffee plants depends on the total amount of photosynthetic active radiation (PAR) received by the plant per day and temporal order of diurnal variations in the PAR supply. That is, plants that received high light in the morning grew faster than those receiving high light in the afternoon. In the second experiment, a trial was designed with two Robusta coffee (*Coffea canephora*) clones that were intercropped with shelter trees in a way that allowed us to compare coffee

bushes that were shaded in the morning (SM) with those shaded in the afternoon (SA), and we compared both treatments with bushes that received full sunlight over the course of the day (FS). The SM bushes displayed better gas exchange performance than their SA and FS counterparts, which means that the capacity for CO_2 fixation was mainly constrained by stomatal (SA bushes) and biochemical (FS bushes) factors. The physiological traits associated with light capture were more responsive to temporal light changes rather than the amount of light received, although this behavior could be a clone-specific response. The activity of key antioxidant enzymes differed minimally when compared between the SM and SA clones but was much greater in the FS clones. No signs of photoinhibition or cell damage were observed regardless of the light treatment. Acclimation to varying light supplies had no apparent additional energy cost for constructing and maintaining the leaves regardless of the light supply. The SM and SA individuals displayed higher returns in terms of revenue streams (e.g., higher mass-based light-saturated photosynthetic rates, photosynthetic nitrogen use efficiencies and long-term water use efficiencies) than their FS counterparts. In summary, shading may improve the physiological performance of coffee bushes that are grown in harsh, tropical environments; however, it is important to select coffee genotypes with adequate phenotypic plasticity to cope with a reduced light supply, as was noted in clone 03.

GENERAL INTRODUCTION

Although light is a crucial environmental resource that affects photosynthesis and ultimately influences plant growth, low and high light can limit plant performance. Shortages of key resources, such as light, can compromise survival and growth; however, plants face heat, desiccation and excessive irradiance under high sunlight (Valladares and Niinimets, 2008). The consequences of variable light environments on plant growth and photosynthesis are currently best understood in the case of sunflecks, in which the duration and frequency of light patches affect carbon assimilation and biomass accumulation through responses to an array of physiological and morphological processes (Valladares and Niinimets, 2008). In crop plants, the effects of different light environments have been examined by comparing plants grown entirely at high light with individuals grown at a fixed level of shade (e.g., using netting with varying degrees of light transmittance) or in agroforestry systems with homogeneous ground cover that varies from sparse to deep shade depending on the shelter tree attributes and management (e.g., crown architecture, spacing and pruning). Thus, local photosynthetically active radiation conditions to which individual leaves are exposed can vary tremendously throughout the canopy of a tree (Niinimets, 2007). Furthermore, the effects of variable light environments are influenced by the temporal scale of diurnal changes in light environments, even when the total amount of light is constant (Sims and Pearcy, 1993; Wayne and Bazzaz, 1993). To the best of our knowledge, no efforts have been made to examine the consequences of temporal diurnal light availability changes on the physiological performance of tropical crop species in the field or in greenhouse trials.

Leaf acclimation to sun and shade conditions through morpho-anatomical and physiological adjustments has been well characterized in a wide range of species (Boardman, 1977; Evans and Poorter, 2001; Lusk *et al*., 2008). Leaves that developed in high light are generally thicker and/or heavier with a higher nitrogen concentration per leaf area, less chlorophyll (Chl) per unit leaf mass with a reduction of Chl *b*, altered chemical composition and construction costs, higher rates of dark respiration and light-saturated photosynthesis and increased photoprotective pigments as well as decreased susceptibility to photosynthesis photoinhibition when compared with their low-light counterparts (Walters, 2005; Niinemets, 2007; Cavatte *et al*., 2012). Whenever absorbed light energy exceeds the capacity of the leaves to use trapped energy through

photosynthesis or to dissipate the energy as heat, photosystem II damage may occur. Protection against excess energy may be achieved through the down-regulation of photochemical efficiency through the xanthophyll cycle or by maintaining electron flux using alternative pathways, such as photorespiration and the Mehler-peroxidase reaction (Ort and Baker, 2002; Logan *et al.*, 2006).

Among agricultural commodities, coffee has a monetary value that is surpassed only by oil. From some 100 species of the *Coffea* genus, only *C. arabica* L. (Arabica coffee) and *C. canephora* Pierre ex A. Froehner (Robusta coffee) are economically important worldwide, and these two species are responsible for approximately 99% of the worldwide coffee production. Robusta trees are generally more vigorous than Arabica trees, and produce more coffee beans with lower production costs and contain approximately twice as much caffeine per bean. Both species evolved in the forest as understory trees; therefore, they are considered to be a shade-demanding species. Most cultivars were derived from wild Arabica populations, such as the germplasm collections of Ethiopia, and they become severely stressed when grown without overhead shade and provide low yields (Van Der Vossen, 1985). However, according to Van Der Vossen (2005), virtually all current cultivars are descendants of early coffee introductions from Ethiopia to Arabia (Yemen), where they were subjected to a relatively dry ecosystem without shade for a thousand years before being introduced to Asia and Latin America. Most of these cultivars have retained the physiological attributes as shade tolerant plants and can respond to various conditions, such as a mild drought and full sunlight. However, some cultivars (e.g. 'Typica') are not suited to the open, showing excessive symptoms of photodamage when grown at full exposure. In any case, modern high-yielding coffee cultivars have been selected in test trials with high external inputs conducted under full sunlight and wide spacing. Hence the performance of Arabica coffee cultivars in full sunlight is likely to have been improved (DaMatta, 2004). Therefore, under intensive crop management, coffee will often produce much higher yields in sunlight than under shade.

Shading has been abandoned as a regular cultural practice in several coffee regions. Even in countries like Colombia, where coffee was predominantly cultivated in the shade until just a few years ago, approximately two-thirds of the crop is grown full sun exposure (DaMatta *et al*., 2007). In Brazil, shading has almost been completely abandoned since the 1950s in response to low crop yields from shaded plantations, which was most likely the result of excessive water competition from other tree species

as well as excessive shading (DaMatta, 2004). However, shaded plantations have various beneficial features, including less sun scorch damage to the berries, greater natural resource conservation, increased biodiversity and greater stability in coffee production. Thus, these shade-grown coffee characteristics have stimulated renewed interest in the use of shade trees, especially in areas where they had previously been eliminated (Beer *et al.*, 1998; DaMatta *et al.*, 2007).

Whether Arabica coffee trees benefit from an association with shelter trees has been disputed for more than a century (Lock, 1888; Beer *et al.*, 1998; DaMatta, 2004; Vander Vossen, 2005; DaMatta *et al*., 2007; Bosselmann *et al*., 2009). For Robusta coffee, this controversy has been virtually ignored, most likely because this species, which constitutes a relatively new commercial crop, has been cultivated in full sunlight conditions since its introduction (1950s-1960s) to such countries as Brazil, where it produces a greater crop yield than Arabica regardless of shade conditions. While a range of studies have been undertaken to examine the effects of shading on the ecophysiology and production of Arabica coffee (for reviews see DaMatta, 2004; DaMatta *et al*., 2010), virtually nothing is known about this subject in Robusta coffee.

Overall, shading (in agroforestry systems) has been recommended for marginal areas when adverse climatic conditions may limit the successful exploitation of a coffee crop. When the agroforestry system is correctly managed [with the proper choice of shade tree species (often deeply rooted species), judicious evaluation of planting density, appropriate frequency of canopy pruning and tree thinning, soil type, water and thermal regimes], water-use efficiency is expected to rise, making the use of shade trees in agroforestry systems a highly recommended option. This practice should translate into obvious productivity advantages for coffee plantations in dry and hot environments (DaMatta, 2004; DaMatta and Ramalho, 2006) provided that shading is not excessive. Nevertheless, it should be emphasized that shading provides little if any benefit to the crop under optimal or near-optimal edaphoclimatic conditions for coffee cultivation; in many cases, it can even be detrimental (Camargo, 1990; Campanha *et al*., 2004; Morais *et al*., 2006).

The protective effects of shading have been associated with a lower radiation input at the coffee canopy level, which may reduce the extent of photooxidative damage, a phenomenon that is frequently observed in coffee grown at full sun exposure in marginal zones, and ultimately increase crop life expectancy (DaMatta, 2004). In addition, the effects from shade trees on coffee physiology are associated with reduced

wind speeds and temperature changes, higher relative humidity and changes in the aerodynamic roughness of the cropped area. Thus, these alterations decrease the leaf-to-air vapor pressure deficit, which in turn allows for longer stomatal opening (favoring CO_2 uptake) without a proportional increase in transpiration rates. Hence, water loss because of excessive crop evapotranspiration should decline, an effect that is enhanced by increased ground cover and a decrease in the abundance of weeds (Maestri *et al*., 2001).

Coffee crops and other species of agricultural interest may be subjected to pronounced temporal diurnal variations in the light supply, as has been empirically noted in agroforestry systems. Even in unshaded plantations, this situation may also be common, such as in plantations in hilly zones. Depending on the relative position of the crop on a hill, the plant may be mostly shaded during the morning or afternoon (with terrain exposure facing west and east, respectively). Empirical observations from the field have shown that when they are shaded in the morning, coffee plant growth (and production) may be depressed relative to plants that receive a more continuous diurnal light supply; an inverse relationship is usually noted in plants under full sunlight in the morning and subjected to some degree of shading in the afternoon. It is believed that these patterns might be associated with increased carbon gains in parallel with higher stomatal aperture in the morning when light is non-limiting (Araújo *et al*., 2008; DaMatta *et al*., 2008; Batista *et al*., 2012). Therefore, our hypothesis is that the physiological performance of a coffee plant is dependent not only on the total amount of light received but also on the temporal scales of diurnal light availability.

In this study, the effects of varying light supply over a range of morphological and physiological traits were investigated in two independent experiments. In the first experiment, Arabica coffee seedlings were grown in pots and observed under the following light treatments: plants grown entirely at three light supplies (100, 40 and 10% sunlight) as well as plants subjected to full sunlight in the morning and shaded (40 and 10% sunlight) in the afternoon, and vice-versa. In the second experiment, a field trial with clones of Robusta coffee intercropped with rubber trees was designed so that the following three light treatments could be established: coffee bushes mostly shaded in the morning and exposed to full sunlight in the afternoon, bushes receiving full sunlight during most of the day and bushes exposed to full sunlight during the morning and shaded in the afternoon.

References

Araújo WL, Dias PC, Moraes GABK, Celin EF, Cunha RL, Barros RS, DaMatta FM. **2008**. Limitations to photosynthesis in coffee leaves from different canopy positions. *Plant Physiology and Biochemistry***46**: 884-890.

Batista KD, Araújo WL, Antunes WC, Cavatte PC, Moraes GAK, Martins SCV, DaMatta FM. **2012**. Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors. *Trees* **26**: 459-468.

Boardman NK. 1977. Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* **28**: 355-377.

Beer J, Muschler R, Kass D, Somarriba E. 1998. Shade management in coffee and cacao plantations. *Agroforestry Systems* **38**: 139-164.

Bosselmann AS, Dons K, Oberthür T, Olsen CS, Raebild A, Usma H. 2009. The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia. *Agriculture, Ecosystem and Environment* **129**: 253-260.

Camargo AP. 1990. A arborização como meio de reduzir as adversidadesclimáticas e promover a sustentação da cafeicultura. In: Proccedings of the 16thCongresso Brasileiro de Pesquisas Cafeeiras. Instituto Brasileiro do Café, Rio de Janeiro, pp. 6-7.

Campanha MM, Santos RHS, Freitas GBD, Martínez HEP, Garcia SLR, Finger FL. 2004. Growth and yield of coffee plants in agroforestry and monoculture systems in Minas Gerais, Brazil. *Agroforestry Systems* **63**:75-82.

Cavatte PC, Rodríguez-López NF, Martins SCV, Mattos MS, Sanglard LMVP, DaMatta FM. **2012.** 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. *Journal of Experimental Botany* **86**: 3071-3082.

DaMatta FM. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. *Field Crops Research* **86**: 99-114.

DaMatta FM, Ramalho JDC. 2006. Impacts of drought and temperature stress on coffee physiology and production: a review. *Brazilian Journal of Plant Physiology* **18**: 55-81.

DaMatta FM, Ronchi CP, Barros RS, Maestri M. 2007. Ecophysiology of coffee growth and production. *Brazilian Journal of Plant Physiology* **19**:485-510.

DaMatta, FM, Cunha RL, Antunes WC, Martins SCV, Araújo WL, Fernie AR, Moraes GABK. 2008. In field-grown coffee trees source-sink manipulation alters photosynthetic rates, independently of carbon metabolism, via alterations in stomatal function. *New Phytologist***178**: 348-357.

DaMatta FM, Ronchi CP, Maestri M, Barros RS. Coffee: environment and crop physiology. In: DaMatta, F.M. (Ed.). Ecophysiology of tropical tree crops. New York: Nova Science Publishers, 2010. p. 181-216.

Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment* **24**: 755-767.

Lock CGW. 1888. Coffee, its culture and commerce in all countries. London: E. & F.N. Spon.

Logan BA, Kornyeyev D, Hardison J, Holaday AS. 2006. The role of antioxidant enzymes in photoprotection. *Photosynthesis Research* **88**: 119-132.

Lusk C, Reich PB, Montgomery RA, Ackerly DD, Cavender-Bares J. **2008**. Why are evergreen leaves so contrary about shade? *Trends in Ecology and Evolution* **23**: 299-303.

Maestri, M.; Barros, RS, Rena AB. **2001**. Coffee. In: Last, F.T. (Ed.). Tree crop ecosystems. Amsterdam: Elsevier Publishers, p. 339-360.

Morais, H, Caramori PH, Ribeiro AMA, Gomes JC, Koguishi MS. 2006. Microclimatic characterization and productivity of coffee plants grown under shade of pigeonpea in Southern Brazil. *Pesquisa Agropecuária Brasileira* **41**: 763-770.

Niinimets U. 2007. Photosynthesis and resource distribution through plant canopies. *Plant, Cell and Environment* **30**: 1052-1071.

Ort DR, Baker NR. 2002. A photoprotective role for O_2 as an alternative electron sink in photosynthesis?. *Current Opinion in Plant Biology* **5**: 193-198.

Sims DA, Pearcy RW. 1993. Sunfleck frequency and duration affects growth rate of the understorey plant, *Alocasia macrorrhiza*. *Functional Ecology* **7:** 683-689.

Valladares F, Niinemets U. **2008**. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics* **39**: 237-257.

Van der Vossen HAM. 1985. Coffee selection and breeding. In: Clifford, M.N.; Willson, K.C. (Eds.). Coffee - botany, biochemistry and production of beans and beverage. London: Crom Helm, pp. 48-96.

Van der Vossen HAM. 2005. A critical analysis of the agronomic and economic sustainability of organic coffee production. *Experimental Agriculture* **41**: 449-473.

Wayne P, Bazzaz FA. 1993. Birch seedling responses to daily time courses of light in experimental forest gaps and shade houses. *Ecology* **74**: 1500-1515.

Walters RG. 2005. Towards an understanding of photosynthetic acclimation. *Journal of Experimental Botany* **56**: 435-447.

CHAPTER 1

Growth and physiological abilities of *Coffea arabica* L. plants to cope with temporal changes in light availability

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Abstract

The coffee plant has traditionally been considered a shade-demanding species, although it performs well without shade and can even out-yield shaded coffee. We hypothesized that the physiological performance and growth of the coffee plant is dependent on the total amount of light received and on temporal variations in light availability. To test this hypothesis, pot-grown Arabica coffee seedlings were subjected to seven light treatments as follows: plants grown entirely under 100%, 40% or 10% sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). The S-100 plants accumulated more biomass with minimal changes in biomass allocation compared to individual plants from other treatments. The increased biomass was apparently independent of photosynthetic rates per unit leaf area as well as the consequential changes in carbohydrate availability; however, the biomass increase was associated with faster leaf area formation with increasing irradiance. In contrast, the lower biomass in the S-10/100 and especially in S-10 individuals should have been a consequence of carbon limitations as well as lower leaf areas. Biomass accumulation depended on the total photosynthetic active radiation (PAR) and on the daily course of PAR supply, as determined by the 35% larger biomass in the S-100/10 individuals relative to the S-10/100 individuals. Changes in photosynthetic rates between the treatments were apparently unrelated to carbohydrate accumulation or photoinhibition. Overall, only minor physiological trait alterations were observed at the leaf level; such changes were

mainly present when comparing the S-10 individuals with those from other treatments. In summary, growth and physiological performance depends on the total amount of PAR received by the plant per day and on the temporal order of diurnal variations in the PAR supply. Thus, plants receiving high amounts of light in the morning grew faster than those receiving high amounts of light in the afternoon.

1. Introduction

Although light is a crucial environmental resource that affects photosynthesis and ultimately influences plant growth, low and high sunlight can limit plant performance. Shortages of key resources, such as light, can compromise growth and survival, whereas plants face heat, desiccation and excessive irradiance in high sunlight (Valladares and Niinemets, 2008). To cope with these stresses, plants have evolved a number of well-known biochemical, physiological and structural changes at the leaf and whole-plant levels that enable them to adjust to a particular set of light conditions (Lusk *et al.*, 2008; Walters, 2005). Many studies of plant light responses have been aimed at unveiling the morphological and physiological mechanisms as well as the ecological implications of tolerance to extremes, such as tolerance to either sun or shade. These responses have often been examined by comparing plants grown entirely at high light with individuals grown at a fixed level of shade (using netting with varying degrees of light transmittance) or plants grown in gaps compared with plants grown in the understory of a forest. Noticeably fewer studies have explored trends in growth and physiological responses to temporal scales of diurnal variations in light availability, particularly in tropical tree crops.

In general, plant growth and biomass allocation patterns at the whole plant level are limited by resource availability, and its evaluation is essential to compare performances under different environmental conditions (Poorter and Sack, 2012). Although the plants may be constrained to different extents within their whole plant allocation pattern, they still tend to show universal plastic responses, such as increasing mass allocation to the leaf plus stem rather than to the roots when growing in the shade and increased allocation to the root relative to the leaf plus stem when growing in soils with a low nutrient or water supply (Poorter *et al*., 2012). Other factors may also strongly affect biomass allocation patterns, such as the growth form, evolved niche and ontogeny (McConhaguay and Coleman, 1999; Poorter and Nagel, 2000; Niklas and

Enquist, 2002). In any case, the patterns of accumulation and biomass allocation in plants that are subjected to temporal variations in light availability throughout the day have been barely explored.

Coffee is one of the most important commodities of international agricultural trade. It evolved in the understory of the African forest and is considered a shade-demanding species. In early plantations, Arabica coffee bushes were planted under taller shade trees to simulate their natural habitat. However, modern coffee cultivars grow well without shade in many situations and even out-yield shaded coffee; therefore, shading has been abandoned as a regular cultural practice in several regions worldwide (DaMatta, 2004; DaMatta *et al.*, 2010). Even in unshaded plantations, the coffee plant may be subjected to pronounced temporal scales of diurnal variations in light supplies, which are frequently found in hilly zone plantations. Depending on the relative crop position on a hill, the plant may be mostly shaded during either the morning or afternoon (with terrain exposure facing west and east, respectively). Empirical observations from the field have shown that when shaded in the morning, the growth (and production) of coffee plants may be depressed relative to plants that receive a more continuous diurnal light supply; an inverse relationship is usually noted in plants grown under full sunlight in the morning and subjected to some degree of shade in the afternoon. We believe that these patterns might be associated with increased carbon gains in combination with higher stomatal conductance in the morning when light is non-limiting (Araújo *et al.*, 2008; Batista *et al.*, 2012; DaMatta *et al.*, 2008). However, the consequences of this type of temporal variation in light supply on photosynthesis and growth has never been tested in coffee or other tropical tree crop species.

We hypothesized that physiological performance, accumulation and biomass allocation in coffee plants are dependent on the total amount of light received and on the temporal scale of diurnal light availability. To test this hypothesis, biomass accumulation and key photosynthetic traits of coffee plants were examined over the following range of light treatments: plants grown entirely at three light exposures (100, 40 and 10% sunlight) as well as plants that were subjected to full sunlight in the morning and shaded (40 or 10% sunlight) in the afternoon and vice-versa.

2. Materials and Methods

2.1. Plant material, growth conditions and experimental design

The experiments were conducted in Viçosa (20˚45'S, 42˚54'W; 650 m a.s.l.) in southeastern Brazil. Uniform coffee seedlings (*Coffea arabica* L. cv 'Red Catuaí IAC 44') were grown from seeds and transplanted after growing three leaf pairs (January 7th, 2010) into 12-L pots containing a mixture of soil, sand and composted manure (4:1:1 v/v/v). After transplantation, the seedlings were randomly submitted to seven light treatments as follows: plants grown entirely under 100%, 40% or 10% sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively) and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). Every day, the shade shelter, which was provided by neutral density black nylon netting, was removed or added at midday according to the treatment. The plants were irrigated and fertilized as required, and no apparent restriction was observed in the root development at the end of the experiment. The pot positions were periodically randomized to minimize any variation within each light environment. The light treatment combinations were applied for 150 days, after which the plants were harvested. The experiment was established in a completely randomized design with eight replicate plants per treatment, and the experimental plot consisted of one individual in each pot. When measurements were made on a single leaf (for photosynthetic parameters), the youngest, most fully expanded leaves from five individuals were used.

Throughout the experiment, the average air temperature was 21.4 ± 0.8°C (the maximum and minimum average temperatures were 27.7°C and 14.7°C, respectively), and the relative humidity was 79.2%, as measured with sensors that were installed at the experimental site. The PAR was measured using three LI-190SA quantum sensors (Li-Cor; Lincoln, NE, USA) that were positioned 1 m above the plant canopies. All 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.

2.2. Morphological traits, biomass accumulation and allocation

At the end of the experiment, various morphological traits [height, total leaf number per plant, single leaf area, number of plagiotropic branches (PB), stem diameter and height/stem diameter ratio (H/D)] were recorded. Total leaf areas were estimated by putting the maximum leaf widths and lengths into the equations described by Antunes *et al.* (2008), which were evaluated in the plants that were subjected to different lighting regimens. In addition, the plants were harvested and separated into orthotropic and plagiotropic branches, leaves and roots. Roots were washed thoroughly with tap water over a 0.5-mm screen sieve. Plant tissues were oven-dried at 70°C for 72 h, after which the dry weights of the leaves, branches and roots were determined. Based on these data, the total biomass (TB), leaf mass fractions (LMF), orthotropic branch mass fractions (OMF), plagiotropic branch mass fractions (PMF), root mass fractions (RMF) and leaf area ratios (LAR) were obtained. In addition, the specific leaf area (SLA) was estimated with 20 leaf discs (14 mm in diameter).

2.3. Photosynthetic measurements

The net rate of carbon assimilation (A), stomatal conductance (g_s) and internal-to-ambient CO_2 concentration ratio (C_i/C_a) were measured in an open system under ambient temperature and CO_2 partial pressure using two cross-calibrated infrared gas analyzers (LI-6400, Li-Cor). Measurements were made during two periods of the day: 0800-1000 h and 1400-1600 h (solar time) under artificial PAR, i.e., 1000, 500 and 130-μmol photons m^{-2} s^{-1} at the leaf level (for 100, 40 and 10% sunlight, respectively). These PAR intensities approximately corresponded to the ambient irradiance that was intercepted by sampled leaves (in their natural angles) for each light treatment at each time point. After fitting the leaf tissue in the leaf chamber, the rates of gas exchange were typically settled within 3-4 min, nearly paralleling the stabilization for internal CO_2 values. The measurements were repeated three times (two at the end of April and one in mid-May) so that the gas-exchange parameters for each replicate were computed as the average values for the measured days.

The chlorophyll *a* fluorescence parameters were determined in the same leaves that were used for the gas exchange measurements with a portable pulse amplitude modulation fluorometer (Mini PAM, Walz; Effeltrich, Germany). Following dark adaptation for 30 min, the leaf tissue was illuminated with a weak modulated measuring

beam (0.03 μmol m^{-2} s^{-1}) to obtain the initial fluorescence (F_0). A saturating white light pulse of 6000 μmol m^{-2} s^{-1} was applied for 0.8 s to ensure maximum fluorescence emission (F_m) from which the variable-to-maximum fluorescence ratio was calculated as $F_v/F_m = [(F_m - F_0)/F_m)]$. The leaf tissue was exposed to actinic PAR (intensities equal to those used for gas exchange measurements) for 60 s to obtain the steady-state fluorescence yield (F_s). A saturating white light pulse (6000 μmol m^{-2} s^{-1}; 0.8 s) was subsequently applied to achieve the maximum light-adapted fluorescence (F_m'). The light-adapted initial fluorescence (F_0') was estimated according to Oxborough and Baker (1997). Using these parameters along with the photochemical (q_P) and non-photochemical (NPQ) quenching coefficients, the actual quantum yield of PSII electron transport (Φ_{PSII}) and apparent electron transport rate (ETR) were calculated as previously described (Chaves *et al.*, 2008).

Photosynthetic light-response curves (*A*/PAR) were produced by increasing the PAR in ten steps from 0 to 1500 μmol m^{-2} s^{-1} at 25°C. The leaf tissues were initially exposed to a 5-Pa CO_2 partial pressure for 5 min to allow stomatal opening; the *A*/PAR curves were subsequently obtained at 40 Pa of CO_2 partial pressure. The dark respiration rates (R_d), light compensation point (LCP), light saturation point (LSP) and light-saturated *A* (A_{max}) were determined from these curves. Further details are presented elsewhere (Cavatte *et al.*, 2012*a*). The responses of *A* to internal CO_2 partial pressure (A/C_i curve) were determined at 1000 μmol (photons) m^{-2} s^{-1} at 25°C. Measurements began at 35-Pa CO_2 partial pressure, and once the steady state was reached, the CO_2 partial pressure was gradually lowered to 5 Pa and increased stepwise to 160 Pa. The maximum rate of carboxylation (V_{cmax}) and maximum rate of carboxylation limited by electron transport (J_{max}) were estimated from these curves, as detailed by Araújo *et al.* (2008). Measurements were made during the early morning in attached leaves.

The fractions of partitioned N in carboxylation (P_r), bioenergetics associated with electron transport (P_b), thylakoid light-harvesting components (P_l) and structural components (P_s) were calculated according to the method of Niinemets and Tenhunen (1997) using the given values for V_{cmax}, J_{max}, SLA and leaf concentrations for chlorophyll and N (see below).

2.4. Biochemical assays

Leaf discs were flash frozen in liquid nitrogen and stored at -80°C until analysis. A 10-mg sample of ground tissue was added to pure methanol, and the mixture was incubated at 70°C for 30 min. After centrifugation (13,000 *g,* 5 min), the hexoses (glucose and fructose) and sucrose in the supernatant were quantified; the concentration of starch was determined from the methanol-insoluble pellet as previously detailed (Praxedes *et al.*, 2006; Ronchi *et al.*, 2006). Total Chl and carotenoids (Car) were extracted using 80% (v/v) aqueous acetone and quantified according to a procedure reported by Lichtenthaler (1987). Total nitrogen (N) was estimated as described in DaMatta *et al.* (1999).

2.5. Statistics

Data were statistically analyzed following a completely randomized design with five to eight replicates. Assumptions of normality and homoscedascity were checked. The data were analyzed by one-way ANOVA, and the means were examined using the Newman-Keuls test at $P \leq 0.05$. All statistical analyses were performed using SAEG software version 9.1 (SAEG, 2007).

3. Results

3.1. Environmental conditions

The incident PARs above the plant canopies were 26.4, 10.5 and 2.9 mol m^{-2} d^{-1} for S-100, S-40 and S-10 individuals, respectively, with values ranging from 13.5 to 19.2 mol m^{-2} d^{-1} over the plant canopies plants that were subjected to changing PAR supplies (Table 1). The plants that were shaded only in the morning received the largest proportion of PAR during this period, and the opposite was true for plants shaded only in the afternoon (Table 1).

3.2. Morphological traits

The morphological traits under study showed a significant response to differences in varying light regimes ($P < 0.05$, Table 2). The greatest reductions in plant

heights were observed in the S-10 treatment with 18% less height compared to the other treatments. The highest leaf numbers were observed in S-100 individuals and decreased to 23% and 71% in the S-40 and S-10 treatments, respectively. In plants that were subjected to temporal variations in light availability, the leaf number in the S-40/100 and S-100/40 plants were similar, whereas the number in S-100/10 individuals was 20% greater than in the S10/100 individuals. The single leaf area was reduced in S-100 relative to its counterparts in the S-40 and S-10 treatments. The S-40/100 and S-100/40 individuals exhibited similar single leaf areas to the plants in S-40. Furthermore, the S-100/10 individuals displayed higher single leaf area than their S-10/100 relatives. Strong decreases in total leaf areas, up to 21% and 64%, were observed in S-40 and S-10 plants, respectively, in comparison to S-100. Minor changes in the total leaf area in S-40/100 and S-100/40 plants and a strong decrease in S-10/100 plants were observed in contrast to their counterparts in S-100/10 plants. Reductions in the PB were observed, particularly in S-10 individuals and not as great in the S-40 and S-100 plants. The PB was remarkably lower in S-10 individuals and did not differ among plants from the other treatments. The stem diameter decreased in relation to light availability, primarily in S-10 individuals and not as much in the S-40 and S-100 individuals. The stem diameters were similar in S-40/100 and S-100/40 plants; however, this trait had lower values in S-10/100 plants compared to the S-100/10 plants. The height/stem diameter ratio (or Slenderness index) was higher in S-10 plants than in S-40 and S-100 plants. This ratio did not differ significantly among plants from the other treatments.

The SLA increased significantly with decreasing PAR for individuals grown at a fixed PAR; for individuals grown under varying PAR, the SLA from S-40/100 and S-100/40 plants was similar to the S-100 plants (~11 m^2 kg^{-1}), whereas for the S-10/100 and S-100/10 plants, the SLA was similar to that of the S-40 plants (~13 m^2 kg^{-1}) (Table 2). Changes in the SLA were mostly responsible for alterations in LAR (Table 2), which ranged from 5.3 m^2 kg^{-1} (S-100) to 10.2 m^2 kg^{-1} (S-10).

3.3. Biomass accumulation and biomass allocation

The total biomass decreased dramatically in plants grown at a fixed level of solar radiation and a reduction in the PAR supply with numbers ranging from 53.8 g in S-100 plants to 9.4 g in their S-10 counterparts (Fig. 1). However, biomass accumulation responded not only to total PAR but also to temporal scales of PAR supply, which was

observed by the larger (52%) biomass in S-100/10 than in S-10/100 individuals despite the small differences in total PAR supply, whereas the differences (18%) in biomass between S-100/40 and S-40/100 did not reach statistical significance. However, greater (35%) biomass was observed in S-100/40 compared to S-40 plants (Fig. 1). Collectively, these results suggest that the amount of PAR received by plants during the morning rather than the amount of light received during the afternoon positively improved growth with a given total daily PAR supply.

Despite the biomass differences, there were no significant alterations in the patterns of biomass allocation among the treatments with two exceptions: (i) the RMF was larger (approximately 20%) in S-100 plants than in S-40 and S-10 individuals (leading to an increased root-to-shoot ratio; data not shown) but similar in plants from other PAR treatments; (ii) the overall proportion of stem biomass invested into plagiotropic (lateral) branches decreased (at the expense of orthotropic branches) with a decreasing PAR supply (Fig. 1).

3.4. Photosynthetic measurements

Gas exchange parameters were assessed under the prevailing PAR availability during their measurements, thus more properly reflecting the differences in actual CO_2 fixation capacity between treatments. In the morning, A did not significantly differ among individuals receiving 40% or 100% sunlight, whereas the absolute lowest A values were found in S-10 and S-10/100 individuals (Table 3). Changes in A were highly correlated with variations in g_s ($r = 0.87$, $P = 0.011$, $n = 35$). Overall, the largest g_s values were observed in S-100 individuals; g_s did not differ significantly between the plants from the other groups. The largest C_i/C_a (0.69) was observed in S-100 and S-10/100 plants, and the lowest values (0.59) were in S-40 plants with intermediate values in the other groups (Table 3). In the afternoon, the A and g_s decreased markedly relative to their values in the morning; these traits, as well as the C_i/C_a, did not differ significantly regardless of the treatment (Table 3).

Irrespective of PAR treatments and time of the day, no signs of photoinhibition were observed because the F_v/F_m ratio remained at high values (~0.80). Overall, the q_P and Φ_{PSII} tended to decrease and NPQ tended to increase with increasing PAR availability in the morning (Table 4). The ETR was markedly lower in S-10 and S-10/100 plants than in other plants, which suggests photochemical limitations to

photosynthesis in those plants (Table 4). These trends were also observed in the afternoon (Table 4). Notably, the highest absolute values of NPQ in the afternoon were observed in plants that were shaded in the morning (S-40/100 and S-10/100 individuals).

The largest A_{max} values were observed in S-100/40 and S-100/10 individuals, the lowest were in S-40 and S-10 individuals and intermediate values were observed for plants with the other PAR treatments (Table 5). The largest LSP values were obtained in plants that were subjected to full sunlight in the morning compared to their counterparts when submitted to some degree of shade. The LCP decreased with decreasing PAR supply in plants grown at a fixed level of PAR, although this trait did not vary consistently among plants that were subjected to varying PAR supplies (Table 5). In general, the changes in LCP paralleled those in R_d (r = 0.89, P = 0.007, n = 35). The V_{cmax} was unresponsive to PAR treatments, whereas the J_{max} was significantly lower in S-10 plants than in plants from the other PAR treatments, which did not differ from one another (Table 5).

Overall, differences in the fractions of N that were allocated into different components of the photosynthetic apparatus (Table 5) were only evident when comparing S-10 individuals with individuals from other PAR treatments. The S-10 plants showed higher P_r and P_l (and lower P_s) values than the other groups (P_b), which suggests a greater N content in the photosynthetic apparatus of the S-10 plants.

3.5. Biochemical assays

Starch and sugar (fructose and glucose) concentrations tended to increase throughout the day, whereas the sucrose concentrations only showed minor changes and were associated with different light regimens, particularly in the plants grown at a fixed level of light (Table 6). Overall, there were no large diurnal differences in starch and soluble sugars among the treatments (Table 6).

The concentrations of Chl and Car and the Chl/N ratio were larger in S-10 plants than in the other PAR treatments when assessed before midday, which did not differ from one another (Table 6). The Chl/Car and Chl *a/b* ratios were unresponsive to the PAR supply (Table 6). No signs of Chl degradation were detected regardless of plant transfer from low-to-high (and vice versa) PAR environments (data not shown), as assessed at the end of the day.

4. Discussion

4.1. Morphological traits, biomass accumulation and allocation

The biomass offers the most direct measure of plant performance as a product of growth (Dawson *et al.*, 2012); therefore, any changes in biomass should offer the clearest indicator of a plant's ability to respond to and take advantage of varying resource (light) availability. We showed that the ability of the coffee plant to produce biomass was significantly and positively dependent on total PAR, as previously suggested by Cavatte *et al.* (2012a). It is unlikely that improved biomass accumulation in high PAR was an exclusive result of higher photosynthetic rates per unit leaf area and, consequently, changes in the carbohydrate availability of leaves during the day, particularly when comparing the S-100 plants with plants grown entirely or partially under 40% sunlight. Rather, the improved biomass in high PAR conditions was mostly associated with increased total leaf areas that appear to be largely related to earlier plagiotropic branch development (higher PMF and PB), which implies that there were more nodes for anchoring the larger number of leaves that are produced with increasing PAR. In contrast, lower biomass accumulation in S-10/100 and especially in S-10 individuals should have been the consequence of carbon limitations as well as light-induced morphogenetic processes related to leaf formation. In agreement with our working hypothesis, biomass accumulation depended on total PAR and on the temporal scales of PAR supply, as determined in a comparison of S-10/100 with S-100/10 individuals. Both groups of individuals received similar total diurnal PAR supplies; however, the larger biomass of the latter should reflect improved *A* (and greater carbohydrate availability) in the long term and be associated with higher PAR in the morning when the environmental conditions are more conducive for higher gas exchange rates (Batista *et al.*, 2012). In addition, leaf formation and expansion may be constrained according to the carbohydrate availability in plants that are subjected to deep shade, which would affect the early phases of leaf development (Pantin *et al.*, 2011) at the whole plant level and was observed in plants grown under the S-10 and S-10/100 treatments.

Changes in biomass allocation in response to varying PAR supplies were relatively small and mostly observed when comparing the extreme treatments, such as the S-100 with S-10 individuals. These results contrast with those of Cavatte *et al.*

(2012*a*), who reported considerable allometric alterations when comparing older coffee saplings grown under 100% or 15% sunlight. Taken together with our results, biomass partitioning in coffee may be dependent on age-related ontogenetic factors (Poorter and Nagel, 2000; Wright and McConnaughay, 2002). Interestingly, all morphological traits under study were virtually unresponsive to temporal scales of PAR supply when comparing S-40/100 with S-100/40 and S-10/100 with S-100/10 individuals. Therefore, any differences in biomass accumulation between those groups are unlikely to have resulted from morphological adjustments (e.g., SLA and RAF), and physiological adjustments (e.g., net assimilation rate) must have played increased roles to explain the differences in biomass, as previously noted when comparing coffee plants grown under 100% or 15% sunlight (Cavatte *et al.*, 2012*a*).

4.2. Photosynthetic performance and acclimation to varying PAR

In the morning, the differences in actual A were largely associated with stomatal factors, although photochemical limitations likely played a role in constraining A in S-10 and S-10/100 individuals, as determined by their low ETR and because their PAR supplies were at a level below their LSP. In the afternoon, the strong decreases in g_s imposed large limitations on A; however, because C_i/C_a did not decrease relative to their morning values, an additional diffusive (mesophyll) limitation or some impairment to CO_2 fixation at the chloroplast level cannot be ruled out (Flexas *et al.*, 2007). Therefore, the decreases in A in the afternoon were apparently unrelated to feedback regulation by carbohydrate accumulation or the photoinhibition of photosynthesis, which is in agreement with previous studies in coffee (Araújo *et al.*, 2008; Batista *et al.*, 2012; DaMatta *et al.*, 2008). The pronounced increases in NPQ, which has been associated with large zeaxanthin pools and a higher de-epoxidation state of the xanthophyll cycle in coffee (Matos *et al.*, 2009; Pompelli *et al.*, 2010) upon transfers from low-to-high PAR coupled with elevated values of F_v/F_m, which suggests that the coffee plants possess well-developed photoprotection mechanisms to cope with excessive radiation loads (Chaves *et al.*, 2008; Moraes *et al.*, 2010; Pompelli *et al.*, 2010).

We noted some differences in A_{max} but not in the V_{cmax} between treatments. Together, these results suggest that the capacity of mesophyll cells to engage in carbon fixation should be greater than necessary to cope with the PAR received by coffee

leaves. These data are in agreement with the results of Araújo *et al.* (2008), which were found in field-grown coffee trees and in line with a suggestion from Laisk *et al.* (2005), which stated that the power of the photosynthetic machinery is over-dimensioned, likely implying an inefficient use of resources.

Overall, only minor physiological acclimations were observed in response to varying PAR supplies in this study. Such differential acclimations were mainly apparent when comparing the S-10 individuals with plants from other treatments. The S-10 individuals displayed some attributes of shade-acclimated leaves, such as low LCP and R_d, and high SLA, Chl and Car pools, a high Chl/N ratio as well as P_r and P_l, which may improve light harvesting and use such that a positive carbon balance may be maintained even under low-PAR conditions (Lusk *et al.*, 2008; Walters, 2005). In summary, our results indicate a poor plasticity of traits, which are believed to cope with the temporal scales of varying PAR. Thus, these results are concordant with those reported by Matos *et al.* (2009), who showed that major phenotypic changes are only observed when comparing deeply shaded with sun-exposed leaves; they also showed that the responses of leaf traits to changing PAR are non-linear and depend on the suite of traits under consideration.

5. Concluding remarks

Despite their characterization as a shade-demanding species, modern coffee cultivars, such as those used in this study, may grow better in full sunlight. This behavior is unlikely to have been directly associated with carbon gains per unit leaf area but rather with light-induced morphogenetic processes related to faster leaf formation, which may lead to increased photosynthetic productivity on a whole-plant level. Even in deep shade (e.g., S-10 individuals) in which carbon gain may obviously limit growth, the biomass accumulation was much more depressed than the actual A per unit leaf area, which lends further support for the key role of light as a major driver in coffee developmental processes. Thus, we demonstrated that biomass accumulation largely depends on the total amount of PAR and on the temporal scales of diurnal variations in PAR supply, such as the amount of PAR that was received by the plants during the morning played a major role in improving the biomass accumulation. This information has practical importance when selecting shelter trees for farms located in hilly zones

where the use of shading should be avoided in terrain exposure facing west if better growth (and production) is a major goal.

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7. References

Antunes WC, Pompelli MF, Carretero DM, DaMatta FM. 2008. Allometric models for non-destructive leaf area estimation in coffee (*Coffea arabica* and *C. canephora*). *Annals of Applied Biology* **153**: 33-40.

Araújo WL, Dias PC, Moraes GABK, Celin EF, Cunha RL, Barros RS, DaMatta FM. 2008. Limitations to photosynthesis in coffee leaves from different canopy positions. *Plant Physiology and Biochemistry* **46**: 884-890.

Batista KD, Araújo WL, Antunes WC, Cavatte PC, Moraes GAK, Martins SCV, DaMatta FM. 2012. Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors. *Trees* **26**: 459-468.

Cavatte PC, Oliveira AAG, Morais LE, Martins SCV, Sanglard LMVP, DaMatta FM. 2012a. Could shading reduce the negative impacts of drought on coffee? A morpho-physiological analysis. *PhysiologiaPlantarum***144**: 111-122.

Cavatte PC, Rodríguez-López NF, Martins SCV, Mattos MS, Sanglard LMVP, DaMatta FM. 2012b. 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. *Journal of Experimental Botany* **63**: 3071-3082.

Chaves ARM, Caten AT, Pinheiro HA, Ribeiro A, DaMatta FM. 2008. Seasonal changes in photoprotective mechanisms of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. *Trees* **22**: 351-361.

DaMatta FM, Amaral JAT, Rena AB. 1999. Growth periodicity in trees of *Coffea arabica* L. in relation to nitrogen supply and nitrate reductase activity. *Field Crops Research* **60**: 223-229.

DaMatta FM. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. *Field Crops Research* **86**: 99-114.

DaMatta FM, Cunha RL, Antunes WC, Martins SCV, Araújo WL, Fernie AR, Moraes GABK. 2008. In field-grown coffee trees source-sink manipulation alters photosynthetic rates, independently of carbon metabolism, via alterations in stomatal function. *New Phytologist***178**: 348-357.

DaMatta FM, Ronchi CP, MaestriM, Barros RS. 2010. Coffee: environment and crop physiology. In: DaMatta FM, ed. *Ecophysiology of tropical tree crops*. New York: Nova Science Publishers, 181-216.

Dawson W, Rohr RP, van Kleunen M, Fischer M. 2012. Alien plant species with a wider global distribution are better able to capitalize on increased resource availability. *New Phytologist***194**: 859-867.

Flexas J, Díaz-Espejo A, Berry JA, Cifre J, Galmes J, Kaldenhoff R, Medrano H, Ribas-Carbó M. 2007. Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. *Journal of Experimental Botany* **58**: 1533-1543.

Laisk A, Eichelmann H, Oja V, Rasulov B, Padu E, Bichele I, Pettai H, Kull O. 2005. Adjustment of leaf photosynthesis to shade in a natural canopy: rate parameters. *Plant, Cell and Environment* **28**: 375-388.

Lichthenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods of Enzymology* **148**: 350-382.

Lusk C, Reich PB, Montgomery RA, Ackerly DD, Cavender-Bares J. 2008. Why are evergreen leaves so contrary about shade? *Trends in Ecology and Evolution* **23**: 299-303.

McConnaughay KD, Coleman JS. **1999**. Biomass allocation in plants: Ontogeny or optimality? A test along three resources gradients. *Ecology* **80**: 2581-2593.

Matos FS, Wolfgramm R, Gonçalves FV, Cavatte PC, Ventrella MC, DaMatta FM. 2009. Phenotypic plasticity in response to light in the coffee tree. *Environmental and Experimental Botany* **67**, 421-427.

Moraes GABK, Chaves ARM, Martins SCV, Barros RS, DaMatta FM. 2010. Why is it better to produce coffee seedlings in full sunlight than in the shade? A morphophysiological approach. *Photosynthetica***48**: 199-207.

Niinemets U, Tenhunen JD. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum. Plant, Cell and Environment* **20**: 845-866.

Niklas KJ, Enquist, BJ. 2002. On the vegetative biomass partitioning of seed plant leaves, stems, and roots. *American.Naturalist.* 159, 482–497.

Oxborough K, Baker NR. 1997. Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of *qP* and F_v'/F_m'without measuring F_0'. *Photosynthesis Research* **54**: 135-142.

Pantin F, Simonneau T, Rolland G, Dauzat M, Muller B. **2011.** Control of leaf expansion: a developmental switch from metabolics to hydraulics. *Plant Physiology* **156**: 803-815.

Pompelli MF, Martins SCV, Antunes WC, Chaves ARM, DaMatta FM. 2010. Photosynthesis and photoprotection in coffee leaves is affected by nitrogen and light availabilities in winter conditions. *Journal of Plant Physiology* **167**: 1052-1060.

Poorter H, Nagel O. 2000. The role of biomass allocation in the growth responses of plants to different levels of light, CO_2 and water, a quantitative review. *Australian Journal of Plant Physiology* **27**: 595-607.

Poorter H, Villar R. 1997. The fate of acquired carbon in plants: chemical composition and construction costs. In: Bazzaz FA, Grace J, eds. *Resource allocation in plants*. San Diego: Academic Press, 39-72.

Poorter H, Pepin S, Rijkers T, DeJong Y, Evans JR, Körner C. 2006. Construction costs, chemical composition and payback time of high- and low-irradiance leaves. *Journal of Experimental Botany* **57**: 355-371.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012. Biomass allocation to leaves stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30-50.

Poorter H, **Sack L. 2012.** Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. *Frontiers in Plants Science* doi: 10.3389/fpls.2012.00259.

Praxedes SC, DaMatta FM, Loureiro ME, Ferrão MAG, Cordeiro AT. 2006. Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. *kouillou*) leaves. *Environmental and Experimental Botany* **56**: 263-273.

Ronchi CP, DaMatta FM, Batista KD, Moraes GABK, Loureiro ME, Ducatti C. 2006. Growth and photosynthetic down-regulation in *Coffea arabica* in response to restricting root volume. *Functional Plant Biology* **33**: 1013-1023.

SAEG. 2007. *Sistema de análisesestatísticas e genéticas*: versão 9.1. Viçosa: Fundação Arthur Bernardes.

Valladares F, Niinemets U. 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics* **39**: 237-257.

Walters RG. 2005. Towards an understanding of photosynthetic acclimation. *Journal of Experimental Botany* **56**: 435-447.

Wright SD, McConnaugghay KD. 2002. Interpreting phenotypic plasticity: The importance of ontogeny. *Plant Species Biology* **17**:19-131.

7. Figures and tables

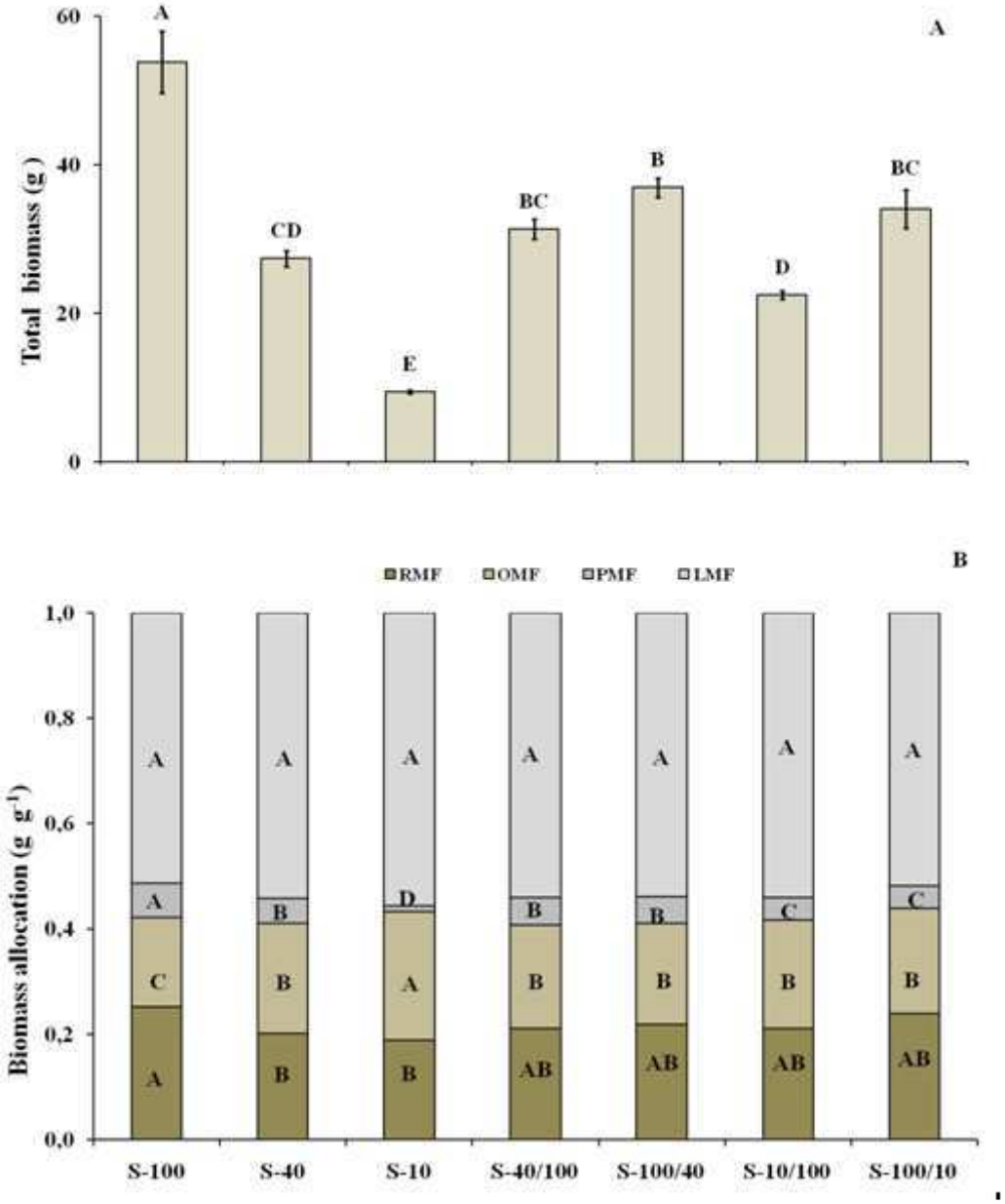

Figure 1. Total biomass (A) and biomass allocation traits (B) [roots mass fraction (RMF), orthotropic branch mass fraction (OMF), plagiotropic branch mass fraction (PMF) and leaf mass fraction (LMF)] in arabica coffee plants subjected to seven light treatments, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-40/100 and S-10/100, respectively). Means followed by a same letter do not differ significantly from one another ($P < 0.05$, Newman-Keuls). n = 8 ± SE.

Table 1. The average incident PAR (mol m^{-2} d^{-1}) and the proportion of PAR from sunrise to midday (PAR_{SR-MD}) and from midday to sunset (PAR_{MD-SS}) over the coffee plants subjected to seven light treatments during 149 days, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). n = 149 ± SE.

Parameters	**Light treatments**						
	S-100	**S-40**	**S-10**	**S-40/100**	**S-100/40**	**S-10/100**	**S-100/10**
PAR	26.4±2.5	10.5±1.1	2.9±0.3	17.7±1.7	19.2±1.9	13.5±1.4	15.8±1.8
	Proportion (%)						
PAR_{SR-MD}	55.2±2.3	55.3±2.1	56.9±1.9	33.4±2.5	75.5±1.4	12.9±1.4	92.0±0.5
PAR_{MD-SS}	44.8±2.3	44.7±2.1	43.1±1.7	66.6±2.5	24.5±1.4	87.1±1.5	8.0±0.4

Table 2. Morphological traits [height (cm), total leaf number per plant, single leaf area (cm^2), total leaf area (m^2),plagiotropic branches number (PBN), stem diameter (mm), height/stem diameter ratio (H/D)] and specific leaf area (SLA, $m^2\ kg^{-1}$) and leaf area ratio (LAR, $m^2\ kg^{-1}$) in arabica coffee plants subjected to seven light treatments, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-40/100 and S-10/100, respectively). Means followed by a same letter do not differ significantly from one another ($P < 0.05$, Newman–Keuls). n = 8 ± SE.

Parameters	Light treatments						
	S-100	S-40	S-10	S-40/100	S-100/40	S-10/100	S-100/10
Height	38.8 ± 1.2^{A}	36.9 ± 1.8^{A}	31.3 ± 0.6^{B}	38.9 ± 0.5^{A}	39.2 ± 1.1^{A}	36.3 ± 0.5^{A}	37.8 ± 1.5^{A}
Leaf number	70 ± 3^{A}	54 ± 2^{B}	20 ± 2^{D}	52 ± 3^{B}	53 ± 2^{B}	43 ± 3^{C}	52 ± 3^{B}
Single leaf area	39.2 ± 1.3^{B}	41.9 ± 1.8^{B}	54.1 ± 3.4^{A}	52.1 ± 3.3^{A}	43.1 ± 2.7^{B}	41.2 ± 1.7^{B}	50.2 ± 2.8^{A}
Total leaf area	0.28 ± 0.02^{A}	0.22 ± 0.01^{B}	0.10 ± 0.01^{D}	0.24 ± 0.02^{AB}	0.27 ± 0.02^{A}	0.17 ± 0.01^{C}	0.24 ± 0.02^{AB}
PB	8.7 ± 0.5^{A}	8.0 ± 0.4^{AB}	2.1 ± 0.3^{C}	7.5 ± 0.3^{AB}	8.0 ± 0.2^{AB}	7.4 ± 0.3^{B}	8.6 ± 0.7^{AB}
Stem diameter	8.3 ± 0.4^{A}	7.5 ± 0.2^{B}	4.7 ± 0.2^{D}	7.3 ± 0.1^{B}	7.6 ± 0.2^{B}	6.6 ± 0.3^{C}	7.6 ± 0.3^{B}
H/D	4.7 ± 0.2^{D}	4.9 ± 0.2^{CD}	6.7 ± 0.2^{A}	5.3 ± 0.1^{BC}	5.2 ± 0.2^{BCD}	5.5 ± 0.2^{B}	5.0 ± 0.1^{CD}
SLA	11.2 ± 0.2^{C}	13.1 ± 0.4^{B}	16.2 ± 0.3^{A}	11.0 ± 0.3^{C}	11.6 ± 0.2^{C}	12.8 ± 0.3^{B}	12.9 ± 0.4^{B}
LAR	5.3 ± 0.3^{C}	8.3 ± 0.6^{B}	10.2 ± 0.6^{A}	7.9 ± 0.5^{B}	7.4 ± 0.4^{B}	7.7 ± 0.4^{B}	7.4 ± 1.0^{B}

Table 3. Gas exchange parameters [net CO_2 assimilation rate (A; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}), internal-to-ambient CO_2 concentration ratio (C_i/C_a; μmol CO_2 mol^{-1} air)] and leaf-to-air vapour pressure deficit (VPD; kPa)] as measured in two time points (08:00-10:00 h and 14:00-16:00 h) in arabica coffee plants subjected to seven light treatments, defined as follows: plants grown entirely under 100%, 40% or 10% full sunlight (S-100, S-40 and S-10, respectively); plants grown at either 40% or 10% full sunlight throughout the morning (until midday) and then submitted to full sunlight until sunset (S-40/100 and S-10/100, respectively); and plants grown under full sunlight from sunrise to midday and then submitted to either 40% or 10% full sunlight throughout the afternoon (S-100/40 and S-100/10, respectively). Means followed by a same letter do not differ significantly from one another ($P < 0.05$, Newman–Keuls test). n = 5 ± SE.

Parameters	Light treatments						
	S-100	**S-40**	**S-10**	**S-40/100**	**S-100/40**	**S-10/100**	**S-100/10**
	8:00-10:00 h						
A	6.3 ± 0.3^{A}	5.0 ± 0.4^{AB}	2.9 ± 0.3^{BCD}	5.1 ± 0.3^{AB}	5.0 ± 0.9^{AB}	2.2 ± 0.2^{D}	5.0 ± 0.4^{AB}
g_s	110 ± 10^{A}	59 ± 5^{BC}	40 ± 5^{C}	78 ± 14^{B}	49 ± 10^{BC}	41 ± 6^{C}	60 ± 5^{BC}
C_i/C_a	0.69 ± 0.02^{A}	0.59 ± 0.01^{B}	0.65 ± 0.01^{AB}	0.64 ± 0.03^{ABC}	0.65 ± 0.02^{ABC}	0.69 ± 0.03^{A}	0.60 ± 0.02^{AB}
VPD	1.9 ± 0.3^{B}	2.3 ± 0.1^{AB}	2.6 ± 0.1^{AB}	2.2 ± 0.2^{AB}	2.6 ± 0.2^{AB}	2.7 ± 0.2^{A}	2.8 ± 0.2^{A}
	14:00-16:00 h						
A	1.2 ± 0.2^{A}	2.1 ± 0.3^{A}	1.2 ± 0.3^{A}	1.3 ± 0.4^{A}	1.4 ± 0.3^{A}	1.5 ± 0.5^{A}	0.7 ± 0.2^{A}
g_s	20 ± 2^{A}	26 ± 3^{A}	20 ± 7^{A}	21 ± 4^{A}	15 ± 4^{A}	16 ± 5^{A}	11 ± 2^{A}
C_i/C_a	0.72 ± 0.02^{A}	0.62 ± 0.04^{A}	0.60 ± 0.05^{A}	0.70 ± 0.02^{A}	0.63 ± 0.04^{A}	0.59 ± 0.04^{A}	0.73 ± 0.02^{A}
VPD	2.4 ± 0.1^{A}	2.1 ± 0.3^{A}	2.7 ± 0.2^{A}	2.5 ± 0.2^{A}	2.9 ± 0.2^{A}	2.9 ± 0.3^{A}	2.8 ± 0.1^{A}

Table 4. Chlorophyll *a* fluorescence parameters [variable-to-maximum fluorescence ratio (F_v/F_m), photochemical quenching coefficient (q_p), non-photochemical quenching coefficient (NPQ), quantum yield of PSII electron transport (Φ_{PSII}) and electron transport rate (ETR)] in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

Parameters	Light treatments						
	S-100	**S-40**	**S-10**	**S-40/100**	**S-100/40**	**S-10/100**	**S-100/10**
				5:00 h			
F_v/F_m	0.79 ± 0.01^{A}	0.79 ± 0.01^{A}	0.80 ± 0.01^{A}	0.79 ± 0.01^{A}	0.79 ± 0.01^{A}	0.80 ± 0.00^{A}	0.78 ± 0.01^{A}
				8:00-10:00 h			
F_v/F_m	0.79 ± 0.02^{A}	0.80 ± 0.00^{A}	0.80 ± 0.00^{A}	0.79 ± 0.01^{A}	0.79 ± 0.01^{A}	0.78 ± 0.01^{A}	0.79 ± 0.01^{A}
q_p	0.18 ± 0.01^{D}	0.40 ± 0.04^{C}	0.75 ± 0.03^{A}	0.41 ± 0.03^{C}	0.17 ± 0.01^{D}	0.63 ± 0.05^{B}	0.23 ± 0.02^{D}
NPQ	1.88 ± 0.05^{AB}	1.70 ± 0.09^{AB}	1.22 ± 0.14^{B}	1.69 ± 0.13^{AB}	2.10 ± 0.12^{A}	1.08 ± 0.10^{B}	2.22 ± 0.14^{A}
ϕ_{PSII}	0.10 ± 0.01^{D}	0.24 ± 0.03^{C}	0.49 ± 0.02^{A}	0.25 ± 0.02^{C}	0.09 ± 0.01^{D}	0.39 ± 0.03^{B}	0.12 ± 0.01^{D}
ETR	52 ± 1^{AB}	63 ± 6^{A}	28 ± 1^{C}	65 ± 5^{A}	47 ± 3^{B}	22 ± 2^{C}	63 ± 4^{A}
				14:00-16:00 h			
F_v/F_m	0.80 ± 0.00^{A}	0.79 ± 0.01^{A}	0.79 ± 0.01^{A}	0.79 ± 0.01^{A}	0.78 ± 0.01^{A}	0.78 ± 0.01^{A}	0.80 ± 0.00^{A}
q_p	0.23 ± 0.05^{C}	0.37 ± 0.03^{B}	0.57 ± 0.01^{A}	0.23 ± 0.06^{C}	0.40 ± 0.03^{B}	0.26 ± 0.09^{C}	0.53 ± 0.01^{A}
NPQ	1.74 ± 0.10^{AB}	1.81 ± 0.16^{AB}	1.43 ± 0.13^{B}	2.55 ± 0.14^{A}	1.05 ± 0.05^{B}	2.46 ± 0.19^{A}	1.19 ± 0.09^{B}
ϕ_{PSII}	0.14 ± 0.03^{C}	0.21 ± 0.02^{B}	0.36 ± 0.04^{A}	0.13 ± 0.01^{C}	0.27 ± 0.03^{AB}	0.13 ± 0.02^{C}	0.35 ± 0.05^{A}
ETR	71 ± 6^{A}	56 ± 4^{A}	20 ± 3^{B}	65 ± 8^{A}	70 ± 7^{A}	67 ± 7^{A}	20 ± 4^{B}

Table 5. Photosynthetic variables derived from the net photosynthetic rates (A) and irradiance curves [light-saturated A (A_{max}; μmol CO_2 m^{-2} s^{-1}), light compensation point (LCP; μmol photons m^{-2} s^{-1}), light saturation point (LSP; μmol photons m^{-2} s^{-1}), and dark respiration (R_d; μmol CO_2 m^{-2} s^{-1})] and from A and internal CO_2 concentration (A/C_i) curves [maximum rate of carboxylation limited by electron transport (J_{max}; μmol e^- m^{-2} s^{-1}) and maximum rate of carboxylation (V_{cmax}; μmol CO_2 m^{-2} s^{-1})], and traits associated with nitrogen partitioning into carboxylation (mainly Rubisco, P_r), bioenergetics (P_b), thylakoid light-harvesting components (P_l) and structural components (P_s) in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

Parameters	Light treatments						
	S-100	**S-40**	**S-10**	**S-40/100**	**S-100/40**	**S-10/100**	**S-100/10**
A_{max}	6.1 ± 0.3^{B}	4.7 ± 0.1^{CD}	4.0 ± 0.3^{D}	5.4 ± 0.3^{BC}	7.2 ± 0.3^{A}	5.6 ± 0.3^{B}	7.2 ± 0.2^{A}
LCP	10.3 ± 0.5^{A}	6.8 ± 1.0^{BC}	3.0 ± 0.4^{D}	9.3 ± 0.5^{AB}	9.4 ± 0.8^{AB}	10.4 ± 1.3^{A}	4.8 ± 0.6^{CD}
LSP	421 ± 27^{A}	275 ± 12^{B}	287 ± 6^{B}	258 ± 14^{B}	376 ± 25^{A}	252 ± 23^{B}	415 ± 15^{A}
R_d	0.78 ± 0.01^{A}	0.49 ± 0.07^{AB}	0.22 ± 0.04^{C}	0.53 ± 0.07^{AB}	0.71 ± 0.12^{A}	0.57 ± 0.11^{AB}	0.41 ± 0.12^{AB}
J_{max}	87.8 ± 4.8^{A}	91.0 ± 1.6^{A}	69.0 ± 8.7^{B}	88.8 ± 1.8^{A}	87.8 ± 3.3^{A}	98.0 ± 1.9^{A}	93.3 ± 1.9^{A}
V_{cmax}	54.5 ± 3.3^{A}	49.7 ± 4.5^{A}	50.3 ± 2.2^{A}	60.2 ± 3.0^{A}	59.3 ± 6.4^{A}	52.3 ± 0.5^{A}	49.7 ± 1.4^{A}
P_r	10.8 ± 0.8^{B}	11.0 ± 0.9^{B}	15.2 ± 0.9^{A}	10.6 ± 0.4^{B}	11.7 ± 1.3^{B}	10.4 ± 0.3^{B}	10.6 ± 0.6^{B}
P_b	1.8 ± 0.1^{AB}	2.1 ± 0.1^{A}	2.1 ± 0.2^{A}	1.6 ± 0.1^{B}	1.8 ± 0.1^{AB}	2.0 ± 0.1^{AB}	2.0 ± 0.1^{AB}
P_l	10.5 ± 0.8^{B}	8.3 ± 1.0^{B}	15.7 ± 0.8^{A}	8.8 ± 0.4^{B}	9.4 ± 0.7^{B}	10.1 ± 0.7^{B}	10.9 ± 0.7^{B}
P_s	76.8 ± 1.0^{A}	78.6 ± 0.3^{A}	66.9 ± 1.3^{B}	79.0 ± 0.6^{A}	77.2 ± 1.7^{A}	77.5 ± 1.0^{A}	76.6 ± 1.3^{A}

Table 6. Leaf starch (g kg^{-1} DW) and soluble sugars concentrations (Glucose+Fructose and Sucrose, mmol kg^{-1} DW) as measured in three time points (5:00, 11:00 and 17:00 h) in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

Parameters	Light treatments						
	S-100	**S-40**	**S-10**	**S-40/100**	**S-100/40**	**S-10/100**	**S-100/10**
				5:00 h			
Starch	93 ± 9^{AB}	68 ± 6^{BC}	52 ± 6^{D}	76 ± 10^{BC}	85 ± 7^{ABC}	76 ± 13^{BC}	117 ± 13^{A}
Glucose+Fructose	28.6 ± 2.2^{A}	23.7 ± 2.2^{A}	24.2 ± 1.2^{A}	32.5 ± 3.1^{A}	32.1 ± 2.5^{A}	29.1 ± 2.4^{A}	29.5 ± 2.0^{A}
Sucrose	227 ± 35^{A}	245 ± 14^{A}	246 ± 24^{A}	254 ± 26^{A}	234 ± 22^{A}	244 ± 21^{A}	221 ± 23^{A}
				11:00 h			
Starch	125 ± 9^{AB}	70 ± 12^{B}	77 ± 15^{B}	115 ± 11^{AB}	124 ± 12^{AB}	85 ± 18^{B}	141 ± 13^{A}
Glucose+Fructose	29.4 ± 2.5^{C}	41.1 ± 3.7^{B}	29.7 ± 1.4^{C}	44.9 ± 2.6^{B}	63.4 ± 5.0^{A}	31.5 ± 2.6^{C}	46.8 ± 1.6^{B}
Sucrose	225 ± 14^{A}	219 ± 18^{A}	291 ± 14^{A}	237 ± 23^{A}	276 ± 11^{A}	225 ± 22^{A}	257 ± 19^{A}
				17:00 h			
Starch	131 ± 10^{A}	134 ± 8^{A}	73 ± 4^{C}	142 ± 13^{A}	136 ± 8^{A}	70 ± 3^{C}	101 ± 7^{B}
Glucose+Fructose	53.5 ± 3.2^{A}	48.1 ± 4.1^{AB}	36.6 ± 1.5^{C}	46.1 ± 3.1^{AB}	51.8 ± 4.3^{A}	35.9 ± 1.9^{C}	47.1 ± 4.1^{AB}
Sucrose	257 ± 11^{A}	238 ± 20^{A}	264 ± 17^{A}	254 ± 23^{A}	257 ± 19^{A}	274 ± 30^{A}	280 ± 27^{A}

Table 7. Leaf concentrations of total chlorophylls (Chl; g kg^{-1} DM) and carotenoids (Car; g kg^{-1} DM)), the ratios of Chl/Car, Chl *a*/*b* and Chl/N (mmol mol^{-1}) in arabica coffee plants subjected to seven light treatments. See further details in the legend of Table 2.

Parameters	Light treatments						
	S-100	**S-40**	**S-10**	**S-40/100**	**S-100/40**	**S-10/100**	**S-100/10**
Chl	10.0 ± 0.9^{B}	8.7 ± 0.9^{B}	14.4 ± 0.7^{A}	9.7 ± 0.3^{B}	9.9 ± 0.6^{B}	10.9 ± 0.6^{B}	10.7 ± 0.8^{B}
Car	2.1 ± 0.1^{B}	1.9 ± 0.2^{B}	2.8 ± 0.1^{A}	2.0 ± 0.1^{B}	2.2 ± 0.1^{B}	2.3 ± 0.1^{B}	2.3 ± 0.1^{B}
Chl/Car	4.7 ± 0.2^{A}	4.5 ± 0.1^{A}	4.7 ± 0.1^{A}	4.8 ± 0.1^{A}	4.6 ± 0.1^{A}	4.7 ± 0.1^{A}	4.6 ± 0.2^{A}
Chl *a*/*b*	2.8 ± 0.1^{A}	2.9 ± 0.1^{A}	2.9 ± 0.1^{A}	2.9 ± 0.1^{A}	3.0 ± 0.1^{A}	2.9 ± 0.1^{A}	2.9 ± 0.1^{A}
Chl/N	3.1 ± 0.3^{B}	2.6 ± 0.3^{B}	4.7 ± 0.3^{A}	2.8 ± 0.1^{B}	2.9 ± 0.2^{B}	3.2 ± 0.2^{B}	3.2 ± 0.2^{B}

CHAPTER 2

Physiological and biochemical abilities of *Coffea canephora* leaves for acclimation to cope with temporal changes in light availability

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Abstract

The effect of varying intensities of light on plants depends on when they occur, even if the total amount of light received is kept constant. We designed an experiment using two clones of robusta coffee (*Coffea canephora*) intercropped with shelters trees in such way that allowed us to compare coffee bushes shaded in the morning (SM) with those shaded in the afternoon (SA), and then confronting both with bushes receiving full sunlight over the course of the day (FS). The SM bushes displayed better gas exchange performance that their SA and FS counterparts, in that the capacity for CO2 fixation was mainly constrained by stomatal (SA bushes) and biochemical (FS bushes) factors. Physiological traits associated with light capture were more responsive to temporal changes of light rather than to the amount of light received, although this behavior could be a clone-specific response. The activity of key antioxidants enzymes differed minimally when comparing the SM and SA clones, but was much larger in FS clones. No signs of photoinibition or cell damage were found regardless of the light treatments. Acclimation to varying light supplies had no apparent additional cost for constructing and maintaining the leaves regardless of the light supply. Both the SM and SA individuals displayed higher return in terms of revenue streams (e.g. higher mass-based light–sutured photosynthetic rates, photosynthetic nitrogen use efficiencies and long-term water use efficiencies) than their FS counterparts. In conclusion, shading may improve the physiological performance of coffee bushes growing in harsh, tropical environments.

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1. Introduction

The effects of variable light environments on plant growth and photosynthesis are a classic topic in plant ecology and forest biology. They are best understood in the case of sunflecks, in which the duration and frequency of light patches affects carbon assimilation and biomass accumulation via responses by an array of physiological and morphological processes (Wayne and Bazzaz, 1993; Pearcy *et al.,* 1994; Valladares and Niinemets, 2008). In crop plants, the effects of light environments have often been examined by comparing plants grown entirely at high light against individuals grown at a fixed level of shade (e.g., using nettings with varying degrees of light transmittance), or in agroforestry systems with more or less homogeneous ground cover, varying from sparse to deep shade, depending on the attributes and management (e.g., crown architecture, spacing, pruning) of the shelter trees. In any case, local photosynthetically active radiation (PAR) conditions to which individual leaves are exposed vary tremendously throughout the canopy of a tree (Niinemets, 2007; Prieto *et al.*, 2012). Furthermore, the effects of variable light environments are also influenced by the temporal scale of the diurnal fluctuations of the light environments, even when the total amount of PAR received is kept constant (Sims and Pearcy, 1993; Wayne and Bazzaz, 1993). To the best of our knowledge, little, if any, no efforts have been undertaken in field conditions to examine the effects of the temporal scale of diurnal changes of light availability in crop species.

Acclimation to sun and shade conditions at the scale of the leaf, via morpho-anatomical and physiological adjustments, has been well characterized in a wide range of species (Boardman, 1977; Evans and Poorter, 2001; Lusk *et al.*, 2008). Leaves developed in high light are generally thicker and/or heavier with a higher nitrogen (N) concentration per leaf area, less chlorophyll (Chl) per unit leaf mass with a reduction of Chl *b*, altered chemical composition and construction costs, higher rates of dark respiration (R_d) and light-saturated photosynthesis (A_{max}), increased photoprotective pigments as well as decreased susceptibility to photoinhibition of photosynthesis compared with their low-light counterparts (Walters, 2005; Niinemets, 2007; Cavatte *et al.*, 2012*b*). Whenever the absorbed light energy exceeds the capacity of leaves to use the trapped energy through photosynthesis or to dissipate it as heat, damage to photosystem II may occur. Protection against excess energy may be achieved by down regulation of photochemical efficiency via the xanthophyll cycle or by maintenance of

the electron flux involving alternative pathways such as photorespiration and the Mehler-peroxidase reaction (Ort and Baker, 2002; Logan *et al.*, 2006).

Among agricultural commodities, coffee, an evergreen tropical shrub crop, has a monetary value surpassed only by oil. Of approximately 100 species of the *Coffea* genus, only *C. arabica* (arabica coffee) and *C. canephora* (robusta coffee) are economically important worldwide. These species have been cultivated in open fields in many tropical countries worldwide despite their origin in shaded habitats (DaMatta, 2004). Presently, there is growing interest in the cultivation of coffee bushes intercropped with shelters trees, due specially to the benefits to shaded plantations, including the conservation of natural resources, increased biodiversity and stability of coffee production in addition to financial benefits, e.g. shelter trees increase cash income from fruits, timber, or latex. In robusta coffee, however, virtually nothing is know on the effects of light supply on its ecophysiology, most likely because this species has been cultivated in full sunlight conditions since its relatively recent introduction (1960s-1970s) in countries such as Brazil.

Under full sunlight, most carbon in robusta coffee is fixed in the morning when the stomatal aperture is higher, paralleling milder vapour pressure deficit (VPD) and temperature conditions (Da Matta *et al.*, 2010). Given this fact, we hypothesized that the physiological performance of robusta coffee could be improved by attenuating the radiation inputs (and temperature) in the afternoon. This could translate into a better local environment for longer stomatal aperture and photosynthetic rates. We further hypothesized that leaves subjected to varying diurnal light supplies should adjust themselves, both morphologically and physiologically, to optimize their photosynthetic performance according to the prevailing temporal scales of diurnal variations of light received by the leaves. To test these hypotheses, we designed an experiment using clones of robusta coffee intercropped with shelter trees in such way that allowed us to compare coffee bushes mostly shaded in the morning with those mostly shaded in the afternoon, and then confronting both with bushes receiving full sunlight over the course of the day. We aimed to examine physiological and biochemical abilities to cope with temporal changes in light supply. Specifically, the carbon gain, the expression of the antioxidant system and chemical composition, construction and maintenance costs of leaves were assessed under real plantation conditions using two clones of robusta coffee with contrasting photosynthetic rates.

2. Material and methods

2.1. Site description, experimental design and growth conditions

The study site is located in the Experimental Station of Sooretama (19°24'S, 40°31W, 30 m elevation), Espírito Santo State, south-eastern Brazil. The soil at the site is a flat, deep, red-yellowish latosol. The site receives an average annual rainfall of 1200 mm mainly distributed from September/October to March/April (the growing season). The average annual temperature is 23.5°C.

The experiment was established in 1999 in an alley cropping system, composed of staggered north-south-oriented rows of rubber trees (cv. 'RRIM 600' – two lines of trees per tree row with a rectangular space of 3.0 m between the lines and 2.5 m between the trees in each line; the tree rows were spaced 40 m from each other) with open land (40 m wide alleys) for coffee bushes (*C. canephora*; Rubiaceae) with 2.5 x 1.0 m spacing in east-west-oriented hedgerows. Thirty-one rows of coffee bushes, each from a single clone, were randomly distributed along the alley. As the sun crosses the sky from east to west over the course of the day, thus perpendicularly crossing the rows of the rubber trees, the shade on the coffee crop migrates accordingly (see Fig. 1). The coffee bushes were evaluated in the following three relative positions within the alley: bushes facing the east rubber tree rows, which were shaded in the morning and were exposed to full sunlight in the afternoon; plants located in the middle of the alley, which received full sunlight during most of the day; and bushes facing the west rows, which were exposed to full sunlight during the morning and were shaded in the afternoon. In summary, the following three light treatments were established: bushes shaded in the morning (SM), bushes under full sunlight (FS) and bushes shaded in the afternoon (SA) (Fig. 1).

The coffee bushes were trained with three orthotropic heads (main stems). Both the rubber trees and coffee bushes were submitted to routine agricultural practices, including hoeing, fertilization and chemical control of insect and pathogen attacks. No supplemental irrigation was provided, but there was abundant rain during the growing season. Sampling and measurements were carried out on cloudless days in January 2010 (the rubber trees were approximately 8 m tall, and the coffee bushes were approximately 2 m tall). Two clones with contrasting photosynthetic performance, that is, clones 03 and 120 displaying relatively higher and lower photosynthetic rates,

respectively (Silva *et al.*, 2012) were analyzed. The evaluations were performed using three bushes per clone per light treatment. In the SM and SA treatments, the bushes near the rubber tree rows (up to 4.0 m from the rows; the coffee bush nearest (1.0 m apart) the tree rows was considered to be the border) in the SM and SA treatments; the three most central bushes in the alley gave the FS treatment (Fig. 1). Two plagiotropic (lateral) branches per bush (each from a distinct orthotropic stem), one facing north and another facing south, were evaluated. The experimental plot consisted of one orthotropic stem per bush. All physiological measurements and leaf samples were taken from the youngest, fully expanded leaves, corresponding to the third or fourth leaf pair from the apex of the branches in the middle third of the coffee bushes.

2.2. Environmental parameters

The total daily PAR over January 2010 was measured using LI-190SA quantum sensors (LI-COR, Lincoln, NE, USA) positioned 1 m above the coffee bushes in each relative position in the alley. Each sensor was precisely positioned above the central bush from the three that were analysed in each light treatment. Air temperature and relative humidity were also monitored. All of the sensors were connected to an LI-1400 data logger (LI-COR), which acquired data from the sensors every minute and stored them as 5-min averages. The leaf-to-air VPD was estimated as described in Chaves *et al.* (2008).

2.3. Photosynthetic parameters

The net rate of carbon assimilation (A), stomatal conductance (g_s), internal-to-ambient CO_2 concentration ratio (C_i/C_a) and transpiration rate (E) were measured in an open system under both ambient temperature and CO_2 partial pressure using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, USA). Instantaneous water use efficiency (WUE), was estimated from the A/E ratio. The variable-to-maximum Chl *a* fluorescence ratio (F_v/F_m) in dark-adapted (30 min) leaves was estimated immediately after gas-exchange analyses using a portable fluorometer (MINI-PAM, Walz, Effeltrich, Germany), as described in Araújo *et al.* (2008). Measurements were made during the following three periods throughout the day: 08:00-10:00 h, 11:00-13:00 h and 14:00-16:00 h (solar time), under artificial PAR, i.e., 250 μmol photons m^{-2} s^{-1} (for the SM

and SA treatments in the morning and afternoon, respectively) and 1250 μmol photons m^{-2} s^{-1} (for the SM and SA treatments in the afternoon and morning, respectively, as well as for FS treatments regardless of time point) at the leaf level. These PAR intensities corresponded approximately to the ambient irradiance intercepted by the sampled leaves (in their natural angles) for each light treatment in each time point. After fitting the leaf tissue in the leaf chamber, the rates of gas exchange were typically settled within 3 min, nearly paralleling the stabilization for internal CO_2 values. The measurements were repeated on three separate days (for each leaf within each time point), such that the gas-exchange parameters for each replicate were computed as the average values obtained over the measurement days.

Photosynthetic light-response curves (A/PAR) were produced by increasing PAR in ten steps from 0 to 1500 μmol m^{-2} s^{-1} at 25°C. Initially, leaf tissues were exposed to a 5 Pa CO_2 partial pressure for 5 min to allow stomatal aperture; subsequently A/PAR curves were obtained at 40 Pa CO_2 partial pressure. Dark respiration rates (R_d), light compensating point (LCP), light saturating point (LSP) and light-saturated A (A_{max}) were determined from these curves. Further details have been given elsewhere (Cavatte *et al.*, 2012*a*). The responses of A to internal CO_2 partial pressure (A/C_i curve) were determined at 1000 μmol (photons) m^{-2} s^{-1}, at 25°C. Measurements started at 35 Pa CO_2 partial pressure and once the steady state was reached, CO_2 partial pressure was gradually lowered to 5 Pa and then increased stepwise up to 160 Pa. The maximum rate of carboxylation (V_{cmax}) and the maximum rate of carboxylation limited by electron transport (J_{max}) were estimated from these curves, as detailed by Araújo *et al.* (2008). Measurements were made in early morning using leaves from branches detached at about 06:00 h, cut submerged in water to prevent embolism of xylem, and immediately brought to the laboratory with their bases immersed in water.

2.4. Chemical composition, construction and maintenance costs of leaf tissues

Leaf tissues were collected at midday, frozen in liquid nitrogen, freeze-dried, ground in a ball mill to allow passage through a 0.080 mm sieve and oven-dried at 60°C for 48 h. 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). From these

values, the carbon isotope composition ratio was estimated, after which the carbon isotope discrimination ($\Delta^{13}C$) was calculated. Further details on this procedure have been reported previously (DaMatta *et al.*, 2003). The proximate chemical composition of the leaves (starch, total soluble sugars, structural carbohydrates plus lignin, lipids, proteins, organic acids, amino acids, minerals, total phenolics and total methylxanthine alkaloids) was determined as described in Poorter and Villar (1997) with the modifications detailed in Cavatte *et al.* (2012*b*), with the exception that total structural carbohydrates (cellulose and hemicellulose) and lignin were quantified together.

The leaf construction costs (CC), defined by the amount of glucose used for constructing one gram of biomass, were estimated using the microbomb calorimeter technique proposed by Williams *et al.*(1987) and described in detail elsewhere (Cavatte *et al.*, 2012*b*). The leaf costs of maintenance (MC) per unit dry mass, which are associated with the energy required to maintain processes that are unrelated to biomass gain, were determined following the procedure reported by Penning de Vries *et al.,* (1974), using the maintenance coefficients reported by Merino *et al.* (1984).

2.5. Biochemical assays

Leaf discs, collected at about midday, were flash frozen in liquid nitrogen and stored at -80ºC until analysis. Total Chl and total carotenoids (Car) were extracted using 80% (v/v) aqueous acetone and quantified according to the procedure reported in Lichtenthaler (1987). Key antioxidant enzymes, including superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6) and glutathione reductase (GR; EC 1.6.4.2), were extracted by grinding with a cold mortar and pestle with polyvinylpolypyrrolidone and appropriate extraction buffers as described in Pinheiro *et al.* (2004).Total SOD activity was determined by measuring its ability to inhibit the photochemical reduction of *p*-nitro-blue-tetrazolium chloride at 560 nm. The activity of CAT was estimated by measuring the rate of decomposition of H_2O_2 at 240 nm; total APX activity was estimated by monitoring the decline in absorbance at 290 nm, and GR activity was assessed by measuring the rate of NADPH oxidation at 340 nm. Further details have been reported previously (Pinheiro *et al.*, 2004). Cellular damage was analysed through malondialdehyde (MDA) accumulation, estimated as the content of total 2-thiobarbituric acid-reactive substances, as detailed in Lima *et al.,* (2002).

2.6. Others measurements

The leaf water potential (Ψ_w) was measured before dawn (04:30-05:30 h) and at midday using a Schollander-type pressure chamber (model 1000, PMS Instruments, Albany, USA). The specific leaf area (SLA; leaf area per unit leaf dry mass) was estimated using 20 leaf discs (each 14 mm in diameter).

2.7. Statistics

The experiment was conducted following a completely randomized design and was analyzed in a factorial (two clones and three light treatments) scheme. The data were analyzed by two-way ANOVA, and the means were compared using the Newman-Keuls and *t* tests at $P \leq 0.05$. All of the statistical analyses were performed using the SAEG System version 9.1 (SAEG, 2007).

3. Results

3.1. Environment

As can be seen in Fig. 2, the total daily PAR over the coffee canopies was 54.0 mol m^{-2} d^{-1} in the FS treatment and decreased by 26.9% and 29.4% in SM and SA treatments, respectively. From sunrise to midday, the SM plants received 11.4 mol photons m^{-2} (44% less than in SA plants), whilst from midday to sunset the SA plants received 12.1 mol photons m^{-2} (43% less than their SM counterparts). For the gas-exchange measurements, the shade provided by the rubber trees was translated into milder microclimatic conditions [lower air (up to 3.0ºC) and leaf (up to 5.7ºC) temperatures and lower leaf-to-air VPD (up to 48%)] than in the full sunlight environments (Table 1). Leaf temperature reached values as high as 42.8ºC, paralleling the elevated leaf-to-air VPD as high as 5.9 kPa, found in clone 120 during the measurements conducted in the early afternoon. Notably, in these measurements, and independent of the clone, both the leaf temperature and leaf-to-air VPD were significantly higher in FS plants than in either SM or SA individuals (Table 1).

3.2. Leaf water potential and specific leaf area

Independent of the clones studied, the Ψ_w before dawn was greater than -0.20 MPa regardless of the light treatments, whereas the Ψ_w at midday was significantly less negative in SM plants than in their FS and SA counterparts, which did not differ from one another in either clone (Table 2).

In clone 03, the SLAs were significantly lower (8%) in FS plants than in their SM and SA counterparts, which did not differ from one another, whereas in clone 120 the SLA of SM and FS plants was similar and lower than in SA individuals (Table 2).

3.3. Gas exchange

Differences in the magnitude of gas exchange between the clones studied were particularly evident in the morning evaluations, at which time clone 03 displayed higher g_s and A values than clone 120 (Table 1). Regardless of the clone and light treatments, the highest g_s values were found in mid-morning, after which g_s decreased progressively throughout the day. Changes in stomatal aperture were closely tracked by changes in A (Table 1), as further highlighting the strong relationship between A and g_s ($r \geq 0.85$; data not shown). Overall, the microclimate alterations caused by the shade around the SM plants in the morning appeared to enable better gas exchange performance (higher g_s and A) in comparison to both SA and FS individuals in both afternoon evaluations. This enhanced performance was translated into a higher diurnal carbon gain in the SM leaves. Despite the differences in both the microclimate conditions and A, the F_v/F_m remained unchanged (≥ 0.76) independent of the treatments (Table 1).

There were no significant differences in LSP among the treatments. In both clones, the LCP did not differ significantly when comparing the FS and SA plants; however, the lowest LCP was found in SM individuals, which was a significant difference in the case of clone 03 (Table 3). The R_d tended to be lower in SM plants when compared with plants from the other light treatments, and again, the difference was significant for clone 03. In this clone, the highest values of A_{max} (both on area and mass bases) were found in SM plants and the lowest in FS plants, with intermediate values in SA individuals, in clone 120, area-based A_{max} was higher in SM than in FS and SA plants, which did not from differ from one another, whereas mass-based A_{max} was similar in SM and SA plants, but higher than in their FS counterparts (Table 3).

Responses of A to C_i (A/C_i curves) reveal that J_{max} was significantly lower (24-30%) in both the SM and FS plants than in their SA counterparts in clone 03; in clone 120 J_{max} responsive to PAR treatments (Table 3).The V_{cmax} did not vary among the light regimens in clone 03; in clone 120, it was lower in FS than in plants from the other treatments (Table 3).

3.4. Photosynthetic pigments, antioxidant enzymes and cellular damages

The SM plants of clone 03 displayed larger concentrations of Chl (63% on average) and Car (25%) and Chl/N ratios (58% on average) than the FS and SA plants, which were similar to one another. The Chl/Car ratio was larger in SM than in FS individuals, with intermediate values in SA plants (Table 4). These pigment parameters were unresponsive to the light treatments in clone 120. Clonal differences were noted in SA plants, but only for the Chl concentration and the Chl/N ratio, which were significantly lower in clone 03 than in clone 120. The Chl a/b ratio remained unchanged (ranging from 2.4 to 2.90) irrespective of treatments (Table 4).

Little, if any, differences in the activity of key antioxidant enzymes were found in either clone when comparing plants from the SM and SA treatments (Table 4). In contrast, increases in the activities of SOD (though not significant in clone 03), APX, GR and particularly in CAT were found in FS plants in both clones, suggesting an augmented oxidative pressure in these plants. However, the MDA concentration was unresponsive to the PAR treatments regardless of the clone analyzed (Table 4).

3.5. Construction and maintenance costs and resource use efficiencies

The CC and MC were unaffected by the treatments (Table 5). This response was accompanied by small, if any, changes in the leaf chemical based in constituents analyzed (Table S1).

In both clones, the PNUE was significantly lower in FS plants than in SM and SA plants. In clone 03, PNUE was higher in SM than SA plants, whereas in clone 120 it did not differ between the SM and SA individuals (Table 5).

In both clones, the daily instantaneous WUE (A/E) did not differ significantly in response to varying PAR conditions. In contrast, relatively large amplitude (18.9 to 23.0$^{o}/_{oo}$) in $\Delta^{13}C$ (a proxy inversely related to long –term WUE) was noted in response

to treatments. In clone 03, $\Delta^{13}C$ was higher in FS plants and lower in SA plants, with intermediate values in SM individuals (Table 5). In clone 120, $\Delta^{13}C$ was higher in FS plants than in their SM and SA counterparts, which did not one other. In either clone, significant correlations ($r \geq 0.57$) between A/E and $\Delta^{13}C$ were found (Fig. S1).

4. Discussion

The experimental design created an environment in which the total, daily integrated PAR was quite similar over the coffee canopies shaded of SM and SA clones, and therefore, we could examine the effects of temporal variations of light availability under similar total daily radiation inputs in the shaded plants (Fig.2). In contrast to our working hypothesis, we found that SM clones displayed better gas exchange performance throughout the day (Table 1). We additionally demonstrated that there were varying abilities for coping with the alterations between the clones we analyzed. This information suggests that considerable phenotypic plasticity may exist in robusta coffee, which may be explored for selecting promising genotypes to be intercropped with shelter trees.

4.1. Gas exchange

The Ψ_w at midday was higher in SM plants than in both FS and SA plants despite the higher g_s (Tables 1 and 2) suggest improved tissue hydration which, together with low leaf temperature and leaf-to-air VPD throughout the morning, should have allowed the SM plants to sustain higher A in comparison with SA and FS plants (Table 1).

The FS plants were subjected to the harshest environmental conditions (high cumulative temperature, leaf-to-air VPD and radiation loads), which could directly impact their A. Despite the strong relationship between A and g_s, we believe that stomatal limitations should not have played a major role in constraining carbon fixation in FS plants. Compelling evidence for this conclusion comes from the fact that the FS individuals from both clones displayed the lowest A_{max} and, in addition, V_{cmax} was also depressed in FS plants relative to SM and SA individuals, as found in clone 120. Notably, the FS plants displayed the highest discrimination against $^{13}CO_2$ (higher $\Delta^{13}C$). Because increases in $\Delta^{13}C$ (which expresses the magnitude of gas exchange over time

instead of a discrete measurement) can arise because of high g_s or low A (Farquhar *et al.*, 1989), it is likely that, in the long term, biochemical limitations at the chloroplast level are the primary constraints to carbon fixation in FS plants.

In the case of SA plants, although A_{max} was lower than in SM plants, both J_{max} and V_{cmax} were kept at high values (Table 3), suggesting that the biochemical capacity for CO_2 fixation was preserved. However, the relatively low *in situ A* suggests that other resources (e.g., water) and environmental conditions were less favorable for carbon gain. In any case, the strong relationship between A and g_s coupled with the absolute lowest $\Delta^{13}C$ values displayed by these plants indicates that stomatal factors played a prominent role in limiting A.

4.2. Antioxidative protection

Considering that carbon fixation, the usual main sink for the absorbed PAR in chloroplasts, was depressed, especially in FS and SA plants and particularly in the afternoon, adjustments in light capture, use and dissipation are required to provide photoprotection to the photosynthetic apparatus. Here, we showed that adjustments in the activity of key antioxidant enzymes (Table 4) associated with alternative pathways for electron flow, such as photorespiration (CAT) and the Mehler-peroxidase reaction (APX, GR) (Logan *et al.*, 2006; Wilhelm and Selmar, 2011), could play important roles in dissipating the excess reducing power. Regardless of both the clone and temporal changes in A, such adjustments were notably responsive to the total amount of PAR received because the enzyme activities differed minimally when comparing the SM and SA plants, but were remarkably larger in FS plants. Additionally, increases in non-photochemical quenching, which has been associated with large zeaxanthin pools and higher pools de-epoxidation state of the xantophyll cycle with increasing light availability in coffee (Matos *et al.*, 2009), may also provide photoprotection trough thermal dissipation (Rodriguez-Calcerrada *et al.*, 2008; Wilhelm and Selmar, 2011). Collectively, these adjustments proved to be sufficient for avoiding photoinhibition and photooxidative damage even under the harsh environmental conditions shown here, as judged from the high F_v/F_m ratio (Table 1) and unchanged MDA concentration (Table 4).

4.3. Acclimation to varying PAR supply: costs and efficiencies

Both CC and MC were virtually unchanged regardless of the clone and PAR treatments (Table 5), probably reflecting the minimal changes in the leaf chemical composition among the treatments (Poorter *et al.*, 2006). In any case, the higher diurnal carbon gain per unit leaf area (with unaltered CC) in SM leaves compared to their FS and SA counterpart suggests a lower time span in which the SM must photosynthesize to recover (amortize) the carbon investment used in their construction (payback time) (Poorter *et al.*, 2006).

We showed that clone 03 was better able than clone 120 to acclimate to temporal alterations of PAR supply to enhance light capture. Such acclimation ability should help clone 03 to optimize the carbon gain when environmental conditions are more conducive for higher rates of gas exchanges, as found in the morning even though the PAR supply is limiting. In this clone, the SM and SA individuals displayed relatively high SLAs (Table 2), which may improve light harvesting per unit of resources invested in construction of photosynthetic tissues (Walters 2005; Lusk *et al.*, 2008); however, the SM plants were better able than the SA plants to acclimate to low PAR via physiological traits, e.g. changes in Chl pools, Chl/N and Chl/ Car ratios (Table 4) which indicate an improved ability for light capture. Furthermore, the SM leaves acclimated to the PAR supply by decreasing both the R_d and LCP (Table 3). Overall, these responses suggest improved light use efficiency when PAR is limiting. Additionally, the SM leaves of clone 03 displayed improved PNUE and higher mass-based A_{max}, which are indicative that, for a give investment (N or biomass), the photosynthetic return is likely to be higher in SM than in SA individuals. Collectively, these acclimations may be interpreted as an evidence of coordinated physiological strategies associated with efficient use of resources in the SM and SA leaves, whereas the SLA was even higher in SA leaves. In any case, both PNUE and mass-based A_{mass} were higher in these kinds of leaves than in their FS counterparts, suggesting impaired resource use efficiency under full sunlight conditions under the present experimental conditions.

The slight differences in instantaneous WUE among the treatments were promptly reflected in $\Delta^{13}C$ (which has been used as an inverse proxy for long-term WUE, Farquhar *et al.*, 1989), a pattern (Fig. S1) consistent with other studies (e.g. Erice *et al*., 2011). Thus, the lowest $\Delta^{13}C$ of FS plants (Table 5) may be assumed as a

compelling evidence of lower long-term WUE of these plants, implying that the FS plants were unable to use the extra PAR received by them. Taking this and all the above information together, our results demonstrate that full sunlight conditions may indeed be detrimental for the efficient use resources by the robusta coffee bushes grown in harsh environments.

5. Conclusions

We demonstrated that shading, particularly in the morning, may improve the physiological performance of coffee bushes growing in a harsh, tropical environment. We also demonstrated that photosynthetic acclimation in response to varying PAR depends on both the clone and nature of the light environment. Importantly, acclimations to varying PAR supplies had not apparent additional costs for constructing and maintaining the leaves regardless of the PAR supply received by them. Overall, both the SM and SA individual displayed higher return in terms of revenue streams (e.g. higher area-and-mass-based A_{max}, PNUE and long-term WUE) than their FS counterparts. Overall, our data lend support for explaining, at least partially, the successful cultivation of coffee bushes intercropped with shelters trees, as has empirically been observed in agroforestry system implanted recently in warm, marginal regions (DaMatta *et al*., 2010). Finally, when adopting intercropping systems, it is important to select coffee genotypes with adequate phenotypic plasticity to cope with reduced light supply, as particularly found in clone 03.

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7. References

Araújo WL, Dias PC, Moraes GABK, Celin EF, Cunha RL, Barros RS, DaMatta FM. 2008. Limitations to photosynthesis in coffee leaves from different canopy positions. *Plant Physiology and Biochemistry* **46**: 884-890.

Boardman NK. 1977. Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* **28**: 355-377.

Cavatte PC, Oliveira AAG, Morais LE, Martins SCV, Sanglard LMVP, DaMatta FM. 2012b. Could shading reduce the negative impacts of drought on coffee? A morphophysiological analysis. *Physiologia Plantarum* **144**: 111-122.

Cavatte PC, Rodríguez-López NF, Martins SCV, Mattos MS, Sanglard LMVP, DaMatta FM. 2012. 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. *Journal of Experimental Botany* **63**: 3071-3082.

Chaves ARM, Caten AT, Pinheiro HA, Ribeiro A, DaMatta FM. 2008. Seasonal changes in photoprotective mechanisms of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. *Trees* **22**: 351-361.

DaMatta FM. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. *Field Crops Research***86**: 99-114.

DaMatta FM, Chaves ARM, Pinheiro HA, Ducatti C, Loureiro ME. 2003. Drought tolerance of two field-grown clones of *Coffea canephora*. *Plant Science* **164**: 111-117.

DaMatta FM, Ronchi CP, Maestri M, Barros RS. 2010. Coffee: environment and crop physiology. In: DaMatta FM, ed. **Ecophysiology of tropical tree crops**. New York: Nova Science Publishers, 181-216.

Erice G, Louahlia G, Irigoyen JJ, Sanchez-Díaz M, Alami T, Avice JC. 2011. Water use efficiency, transpiration and net gas exchange of four alfalfa genotypes submitted to progressive drought and subsequent recovery. *Environmental Experimental Botany* **72**:123-130.

Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment* **24**: 755-767.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503-537.

Lichthenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methodsof Enzymology* **148**: 350-382.

Lima ALS, DaMatta FM, Pinheiro HA, Totola MR, Loureiro ME. 2002. Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. *Environmental and Experimental Botany* **47**: 239-247.

Logan BA, Kornyeyev D, Hardison J, Holaday AS. 2006. The role of antioxidant enzymes in photoprotection. *Photosynthesis Research* **88**: 119-132.

Lusk C, Reich PB, Montgomery RA, Ackerly DD, Cavender-Bares J. 2008. Why are evergreen leaves so contrary about shade? *Trends in Ecology and Evolution* **23**: 299-303.

Mattos FS, Wolfrang R, Gonçalves FV, Cavatte PC, Ventrella MC, DaMatta FM. 2009. Phenotypic plasticity in response to light in the coffee tree. *Enviromental Experimental Botany* **67**: 421-427.

Merino JA, Field CB, Mooney HA. 1984. Construction and maintenance costs of Mediterranean-climate evergreen and deciduous leaves. II. Biochemical pathway analysis. *Acta Oecologica* **5**: 211-229.

Niinemets U. 2007. Photosynthesis and resource distribution through plant canopies. *Plant Cell and Environment* **30**: 1052-1071.

Ort DR, Baker NR.2002. A photoprotective role for O_2 as an alternative electron sink in photosynthesis? *Current Opinion in Plant Biology* **5**: 193-198.

Pearcy RW, Chazdon RL, Gross LJ, Mott KA. 1994. Photosynthetic utilization of sunflecks: a temporally patchy resource on a time scale of seconds to minutes. In: Caldwell MM, Pearcy RW, eds. *Exploitation of environmental heterogeneity by plants*. San Diego: Academic Press, 175-208.

Penning de Vries FWT, Brunsting AHM, van Laar HH. 1974. Products, requirements and efficiency of biosynthesis: a quantitative approach. *Journal of Theoretical Biology* **45**:339-377.

Pinheiro HA, DaMatta FM, Chaves ARM, Fontes EPB, Loureiro ME. 2004. Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. *Plant Science* **167**: 1307-1314.

Poorter H, VillarR. 1997. The fate of acquired carbon in plants: chemical composition and construction costs. In: Bazzaz FA, Grace J, eds. *Resource allocation in plants*. San Diego: Academic Press, 39-72.

Poorter H, Pepin S, Rijkers T, DeJong Y, Evans JR, Körner C. 2006. Construction costs, chemical composition and payback time of high- and low-irradiance leaves. *Journal of Experimental Botany* **57**: 355-371.

Prieto JA, Louarn G, Perez-Peña J, Ojeda H, Simonneau T, Lebon E. 2012. A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera* L.). *Plant, Cell and Environment* doi: 10.1111/j.1365-3040.2012.02491.x.

SAEG. 2007. *Sistema de análises estatísticas e genéticas*: versão 9.1. Viçosa: Fundação Arthur Bernardes.

Sims DA, Pearcy RW. 1993. Sunfleck frequency and duration affects growth rate of the understory plant, *Alocasia macrorrhiza. Functional Ecology* **7**: 683-689.

Valladares F, Niinemets U. 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics* **39**: 237-257.

Walters RG. 2005. Towards an understanding of photosynthetic acclimation. *Journal of Experimental Botany* **56**: 435-447.

Wayne P, Bazzaz FA. 1993. Birch seedling responses to daily time courses of light in experimental forest gaps and shadehouses. *Ecology* **74**: 1500-1515.

Wilhelm C, Selmar D. 2011. Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. *Journal of Plant Physiology* **168**: 79-87.

Williams K, Percival F, Merino J, Mooney HA. 1987. Estimation of tissue construction cost from heat of combustion and organic nitrogen content. *Plant, Cell and Environment* **10**: 725-734.

8. Tables and figures

Table 1. The air (T_A; ºC) and leaf (T_L; ºC) temperatures, leaf-to-air vapour pressure deficit (VPD; kPa), net CO_2 assimilation rate (A;µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}), internal-to-ambient CO_2 concentration ratio (C_i/C_a; µmol CO_2 mol^{-1} air) and variable-to-maximum Chl fluorescence ratio (F_v/F_m), as measured in three time points at approximately 08:00-10:00 h, 11:00-1300 h and 14:00-16:00 h in two clones of robusta coffee subjected to three light treatments, defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Within each clone, capital letters denote significant differences among light treatments; within each light treatment, small letters denote significant differences between clones ($P \leq 0.05$. (Newman–Keuls' and t-tests). $n = 6 \pm$ SE

Parameters	Clone 03			Clone 120		
	SM	FS	SA	SM	FS	SA
			08:00-10:00 h			
T_A	35.4±0.6^{Ba}	37.4±0.8^{Aa}	38.5±0.1^{Aa}	35.4±0.6^{Ba}	37.4±0.8^{Aa}	38.5±0.1^{Aa}
T_L	33.9±0.5^{Cb}	37.8±0.5^{Cb}	39.6±0.1^{Aa}	35.5±0.4^{Ba}	39.6±0.3^{Aa}	39.9±0.1^{Aa}
VPD	2.1±0.2^{Cb}	3.4±0.2^{Bb}	4.0±0.0^{Aa}	2.9±0.2^{Ba}	4.2±0.1^{Aa}	4.2±0.1^{Aa}
A	6.3±0.14^{Aa}	6.1±0.13^{Aa}	5.8±0.18 ^{Aa}	5.2±0.21^{Ab}	2.9±0.1^{Cb}	3.7±0.1^{Bb}
g_s	160±8^{Aa}	96±3 ^{Ba}	80±4 ^{Ba}	122±13^{Ab}	40±2^{Bb}	52±3^{Bb}
E	3.5±0.2^{Aa}	3.3±0.2^{Aa}	3.2±0.1^{Aa}	2.9±0.0^{Aa}	1.7±0.0^{Cb}	2.3±0.1^{Bb}
C_i/C_a	0.77±0.01^{Aa}	0.66±0.01^{Ba}	0.62±0.01^{Ba}	0.74±0.01^{Aa}	0.63±0.01^{Ba}	0.63±0.01^{Ba}
F_v/F_m	0.83±0.00^{Aa}	0.81±0.01^{ABa}	0.80±0.01^{Ba}	0.81±0.01^{Aa}	0.77±0.02^{Aa}	0.80±0.01^{Aa}
			11:00-13:00 h			
T_A	37.9±0.1^{Ca}	40.4±0.1^{Aa}	39.6±0.1^{Ba}	37.9±0.1^{Ca}	40.4±0.1^{Aa}	39.6±0.1^{Ba}
T_L	39.9±0.2^{Ba}	41.6±0.1^{Ab}	38.8±0.1^{Cb}	38.9±0.2^{Cb}	42.8±0.3^{Aa}	40.7±0.2^{Ba}
VPD	4.5±0.1^{Ba}	5.3±0.0^{Ab}	4.5±0.0^{Bb}	4.2±0.2^{Ca}	5.9±0.1^{Aa}	5.0±0.1^{Ba}
A	5.2±0.1^{Aa}	2.5±0.2^{Ba}	1.7±0.1^{Cb}	4.9±0.9^{Aa}	1.9±0.1^{Cb}	3.0±0.2^{Ba}
g_s	65±2^{Aa}	31±2^{Ba}	19±1^{Cb}	63±5^{Aa}	24±1^{Cb}	37±2^{Ba}
E	2.9±0.0^{Aa}	1.7±0.5^{Ba}	0.9±0.0^{Cb}	2.6±0.1^{Ab}	1.6±0.1^{Ba}	1.8±0.1^{Ba}
C_i/C_a	0.59±0.01^{Aa}	0.59±0.00^{Aa}	0.56±0.01^{Ab}	0.61±0.00^{Aa}	0.61±0.01^{Aa}	0.61±0.01^{Aa}
F_v/F_m	0.82±0.00^{Aa}	0.81±0.00^{Aa}	0.82±0.00^{Aa}	0.82±0.00^{Aa}	0.79±0.01^{Ba}	0.82±0.00^{Aa}
			14:00-16:00 h			
T_A	35.8±0.5^{Cb}	34.3±0.4^{Cb}	32.5±0.4^{Aa}	35.8±0.5^{Cb}	34.3±0.4^{Cb}	32.5±0.4^{Aa}
T_L	37.4±0.6^{Aa}	36.1±0.4^{Ab}	32.1±0.0^{Bb}	37.4±0.0^{Aa}	34.6±0.8^{Bb}	33.3±0.0^{Ca}
VPD	4.1±0.2^{Aa}	3.6±0.1^{Ba}	2.4±0.0^{Ca}	4.0±0.0^{Aa}	3.2±0.3^{Ba}	2.7±0.0^{Ca}
A	1.4±0.2^{Aa}	1.0±0.1^{Aa}	0.5±0.1^{Ba}	1.5±0.6^{Aa}	0.9±0.1^{Ba}	0.8±0.1^{Ba}
g_s	16±1.9^{Aa}	16±0.0^{Aa}	8 ±0.7^{Bb}	22±0.1^{Aa}	17±0.2^{Ba}	12±0.2^{Ca}
E	0.8±0.1^{Aa}	0.6±0.1^{Aa}	0.2±0.0^{Ba}	0.9±0.1^{Aa}	0.6±0.1^{Ba}	0.3±0.0^{Ca}
C_i/C_a	0.78±0.10^{Aa}	0.69±0.01^{Aa}	0.70±0.01^{Aa}	0.81±0.06^{Aa}	0.71±0.01^{Aa}	0.68±0.01^{Aa}
F_v/F_m	0.79±0.03^{Aa}	0.76±0.02^{Aa}	0.81±0.01^{Aa}	0.79±0.05^{Aa}	0.79±0.01^{Aa}	0.81±0.01^{Aa}

Table 2. The leaf water potential at predawn (Ψ_{pd}; MPa) and midday (Ψ_{md}; MPa) and the specific leaf area (SLA; $m^2\ kg^{-1}$) in two clones of robusta coffee subjected to three light treatments, defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1

Parameter	Clone 03			Clone 120		
	SM	FS	SA	SM	FS	SA
Ψ_{pd}	-0.14±0.02^{Ab}	-0.14±0.03^{Aa}	-0.17±0.03^{Aa}	-0.07±0.01^{Aa}	-0.14±0.03^{Ba}	-0.19±0.04^{Ba}
Ψ_{md}	-0.84±0.06^{Aa}	-1.23±0.08^{Ba}	-1.17±0.05^{Ba}	-0.92±0.07^{Aa}	-1.32±0.05^{Ca}	-1.19±0.05^{Ba}
SLA	12.8±0.3^{Aa}	11.7±0.2^{Ba}	12.7±0.3^{Aa}	11.3±0.2^{Bb}	10.7±0.4^{Bb}	12.8±0.3^{Aa}

Table 3. Photosynthetic variables derived from the net photosynthetic rates (A) and irradiance curves [light compensation point (LCP; µmol photons m^{-2} s^{-1}), light saturation point (LSP;µmol photons m^{-2} s^{-1}), light-saturated A (A_{max}; µmol CO_2 m^{-2} s^{-1}) and dark respiration (R_d; µmol CO_2 m^{-2} s^{-1})] and from A and internal CO_2 concentration (A/C_i) curves [maximum rate of carboxylation limited by electron transport (J_{max}; µmol e^- m^{-2} s^{-1}) and maximum rate of carboxylation (V_{cmax}; µmol CO_2 m^{-2} s^{-1})] and carbon isotope discrimination ($\Delta^{13}C$; ‰) in two clones of robusta coffee subjected to three light treatments, defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

Parameters	Clone 03			Clone 120		
	SM	FS	SA	SM	FS	SA
LCP	10.7 ± 0.3^{Bb}	27.6 ± 0.3^{Aa}	20.7 ± 0.3^{Ca}	18.7 ± 1.8^{Aa}	24.8 ± 1.5^{Aa}	20.7 ± 1.2^{Aa}
LSP	599 ± 52^{Aa}	567 ± 45^{Aa}	660 ± 29^{Aa}	552 ± 47^{Aa}	560 ± 15^{Aa}	582 ± 447^{Aa}
R_d	1.0 ± 0.1^{Ba}	1.4 ± 0.2^{Aa}	1.4 ± 0.2^{Aa}	1.3 ± 0.1^{Aa}	1.6 ± 0.1^{Aa}	1.4 ± 0.1^{Aa}
$A_{max\ (area)}$	10.7 ± 0.3^{Aa}	7.2 ± 0.2^{Ca}	8.8 ± 0.1^{Ba}	9.2 ± 0.5^{Aa}	7.0 ± 0.5^{Ba}	7.8 ± 0.1^{Bb}
$A_{max\ (mass)}$	138 ± 6^{Aa}	85 ± 3^{Ca}	110 ± 5^{Ba}	103 ± 4^{Ab}	76 ± 6^{Ba}	100 ± 4^{Aa}
J_{max}	73.3 ± 7.4^{Bb}	72.1 ± 8.5^{Ba}	102 ± 5.5^{Aa}	100 ± 3.8^{Aa}	76.3 ± 8.9^{Aa}	100 ± 7.7^{Aa}
V_{cmax}	81.3 ± 11.7^{Ab}	89.6 ± 2.9^{Aa}	91.0 ± 4.8^{Aa}	109 ± 5.7^{Aa}	79.1 ± 4.4^{Ba}	98.0 ± 3.7^{ABa}

Table 4. Leaf concentrations of total chlorophylls (Chl; g kg^{-1} DM) and carotenoids (Car; g kg^{-1} DM) and the ratios of Chl/N (mmol mol^{-1}), Chl/Car and Chl *a*/*b* in two clones of robusta coffee subjected to three light treatments defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

Parameters	Clone 03			Clone 120		
	SM	FS	SA	SM	FS	SA
Chl	11.1 ± 1.3^{Aa}	6.7 ± 0.3^{Ba}	7.2 ± 0.3^{Bb}	9.9 ± 0.7^{Aa}	8.0 ± 0.7^{Aa}	9.7 ± 0.5^{Aa}
Car	2.0 ± 0.1^{Aa}	$1.5\pm0.1A^{Ba}$	1.5 ± 0.2^{Ba}	1.9 ± 0.2^{Aa}	1.7 ± 0.2^{Aa}	1.9 ± 0.2^{Aa}
Chl/ N	5.2 ± 0.6^{Aa}	3.2 ± 0.2^{Ba}	3.4 ± 0.1^{Bb}	4.8 ± 0.2^{Aa}	3.9 ± 0.3^{Aa}	4.5 ± 0.3^{Aa}
Chl/Car	5.5 ± 0.3^{Aa}	4.4 ± 0.1^{Ba}	4.9 ± 0.1^{ABa}	5.4 ± 0.4^{Aa}	4.9 ± 0.2^{Aa}	5.4 ± 0.4^{Aa}
Chl *a*/*b*	2.5 ± 0.2^{Aa}	2.9 ± 0.1^{Aa}	2.9 ± 0.1^{Aa}	2.4 ± 0.1^{Aa}	2.8 ± 0.1^{Aa}	2.6 ± 0.2^{Aa}
SOD	1.1 ± 0.1^{Ba}	1.5 ± 0.1^{Aa}	1.2 ± 0.1^{ABa}	1.1 ± 0.1^{ABa}	1.4 ± 0.1^{Aa}	1.0 ± 0.1^{Ba}
APX	22.4 ± 3.0^{Ba}	36.2 ± 4.3^{Ab}	23.8 ± 2.4^{Ba}	30.9 ± 3.4^{Ba}	45.7 ± 2.4^{Aa}	31.4 ± 2.7^{Ba}
GR	1.8 ± 0.1^{Ba}	2.7 ± 0.1^{Aa}	1.8 ± 0.2^{Ba}	1.6 ± 0.2^{Ba}	3.1 ± 0.2^{Aa}	1.9 ± 0.1^{Ba}
CAT	1.2 ± 0.1^{Ba}	2.9 ± 0.1^{Ab}	1.3 ± 0.1^{Ba}	1.1 ± 0.2^{Ba}	4.3 ± 0.1^{Aa}	1.3 ± 0.1^{Ba}
MDA	32.1 ± 1.7^{Aa}	32.7 ± 1.3^{Aa}	31.8 ± 1.5^{Aa}	35.2 ± 1.2^{Aa}	32.7 ± 1.1^{Aa}	33.0 ± 1.0^{Aa}

Table 5. The construction (CC; g glucose g^{-1} DM) and maintenance (MC; mg glucose g^{-1} DM day^{-1}) costs, photosynthetic nitrogen use efficiency (PNUE; μmol CO_2 $g^{-1}N$ s^{-1}),average daily instantaneous water use efficiency (*A/E*; mmol mol^{-1}) and carbon isotope discrimination ($\Delta^{13}C$; ‰) in two clones of robusta coffee subjected to three light treatments defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

Parameters	Clone 03			Clone 120		
	SM	FS	SA	SM	FS	SA
CC	1.28 ± 0.02^{Aa}	1.33 ± 0.02^{Aa}	1.29 ± 0.02^{Aa}	1.28 ± 0.0^{Aa}	1.29 ± 0.02^{Aa}	1.28 ± 0.03^{Aa}
MC	16.1 ± 0.4^{Aa}	15.7 ± 0.3^{Aa}	16.3 ± 0.3^{Aa}	16.9 ± 0.6^{Aa}	15.9 ± 0.2^{Aa}	16.6 ± 0.3^{Aa}
PNUE	4.56 ± 0.07^{Aa}	2.92 ± 0.07^{Ba}	3.72 ± 0.06^{Ca}	3.49 ± 0.08^{Ab}	2.59 ± 0.01^{Aa}	3.37 ± 0.03^{Ba}
A/*E*	1.98 ± 0.18^{Aa}	1.80 ± 0.23^{Aa}	2.13 ± 0.22^{Aa}	1.82 ± 0.14^{Aa}	1.67 ± 0.25^{Aa}	1.95 ± 0.23^{Aa}
$\Delta^{13}C$	21.0 ± 0.5^{Ba}	23.0 ± 0.2^{Aa}	19.4 ± 0.1^{Ca}	19.5 ± 0.5^{Bb}	21.8 ± 0.2^{Ab}	18.9 ± 0.1^{Ba}

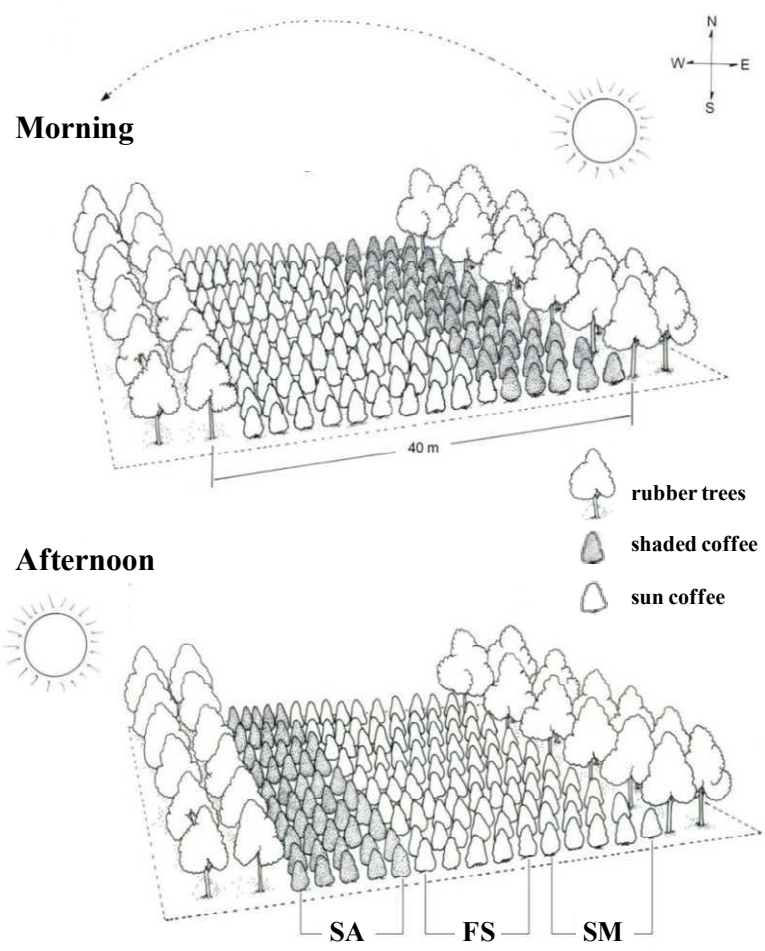

Fig. 1. Schematic representation showing an alley cropping system, composed of north-south-oriented rows of rubber trees (two lines of trees per row; the tree rows were spaced 40 m from each other) with open land (40 m wide alleys) for coffee bushes growing in east-west-oriented hedgerows. As the sun crosses the sky from east to west over the course of the day, thus perpendicularly crossing the rows of the rubber trees, the shade on the coffee crop migrates accordingly. Three light treatments were established, defined as follows: bushes facing the east rubber tree rows, which were shaded in the morning and exposed to full sunlight in the afternoon (SM); plants located in the middle of the alley, receiving full sunlight during most of the day (FS); and bushes facing the west rows, which were exposed to full sunlight during the morning and shaded in the afternoon (SA).

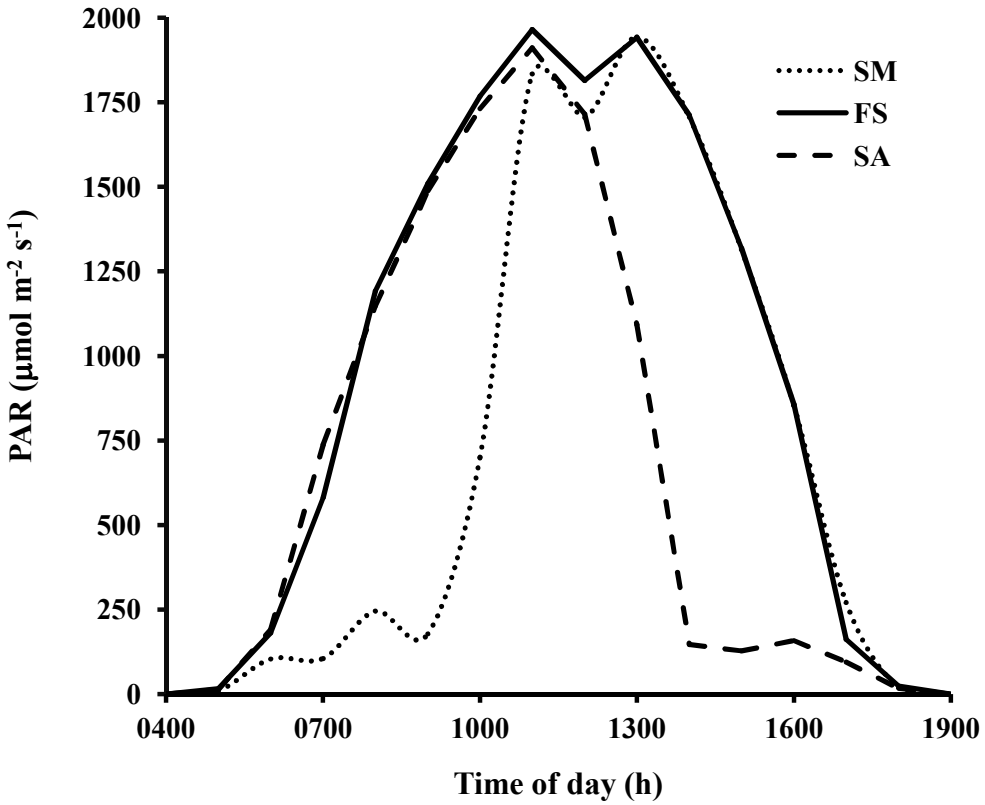

Fig. 2. The time course of the photosynthetically active radiation (PAR) over the coffee canopies. Three PAR treatments were established, defined as follows: coffee bushes shaded in the morning and exposed to full sunlight in the afternoon (SM); bushes receiving full sunlight during most of the day (FS); and bushes facing the west rows, which were exposed to full sunlight during the morning and shaded in the afternoon (SA).

Table S1. The chemical composition (expressed in g kg^{-1} DM) of leaf tissues [starch, total soluble sugars (TSS), structural carbohydrates plus lignin (SCL), proteins, lipids, organic acids (OA), amino acids (AA), minerals, phenols and alkaloids] and the construction (CC; g glucose g^{-1} DM) and maintenance (MC; mg glucose g^{-1} DM day^{-1}) costs in two clones of robusta coffee subjected to three light treatments defined as follows: clones shaded in the morning and exposed to full sunlight in the afternoon (SM); clones receiving full sunlight during most of the day (FS); and clones exposed to full sunlight during the morning and shaded in the afternoon (SA). Statistics are defined as in Table 1.

Parameters	Clone 03			Clone 120		
	SM	FS	SA	SM	FS	SA
Starch	150 ± 1^{Aa}	146 ± 1^{Aa}	151 ± 1^{Aa}	163 ± 1^{Aa}	114 ± 2^{Bb}	151 ± 1^{Aa}
TSS	76 ± 3^{Aa}	77 ± 4^{Ab}	62 ± 7^{Bb}	88 ± 3^{ABa}	78 ± 1^{Aa}	85 ± 6^{Aa}
SCL	290 ± 30^{Aa}	239 ± 7^{Aa}	254 ± 26^{Aa}	207 ± 41^{Ab}	220 ± 16^{Aa}	282 ± 28^{Aa}
Proteins	84 ± 10^{Ab}	79 ± 5^{Ba}	72 ± 4^{Ba}	101 ± 10^{Aa}	79 ± 4^{Ba}	87 ± 11^{ABa}
Lipids	107 ± 3^{Bb}	107 ± 3^{Ba}	130 ± 6^{Aa}	127 ± 7^{Aa}	110 ± 5^{Aa}	127 ± 8^{Aa}
AO	81 ± 5^{Ab}	89 ± 6^{Aa}	86 ± 4^{Aa}	94 ± 4^{Aa}	80 ± 5^{Ba}	74 ± 4^{Ba}
AA	7.4 ± 0.3^{Aa}	7.8 ± 0.2^{Aa}	8.1 ± 0.3^{Aa}	7.8 ± 0.3^{Aa}	8.3 ± 0.4^{Aa}	8.2 ± 0.2^{Aa}
Minerals	52 ± 6^{Aa}	41 ± 3^{Aa}	48 ± 4^{Aa}	41 ± 3^{ABa}	35 ± 4^{Ba}	49 ± 3^{Aa}
Phenols	140 ± 14^{Ab}	140 ± 13^{Ab}	173 ± 12^{Aa}	213 ± 14^{Aa}	205 ± 6^{ABa}	180 ± 18^{Ba}
Alkaloids	21 ± 1^{Ab}	26 ± 1^{Ab}	20 ± 2^{Ab}	30 ± 3^{Ba}	38 ± 3^{Aa}	29 ± 2^{Ba}

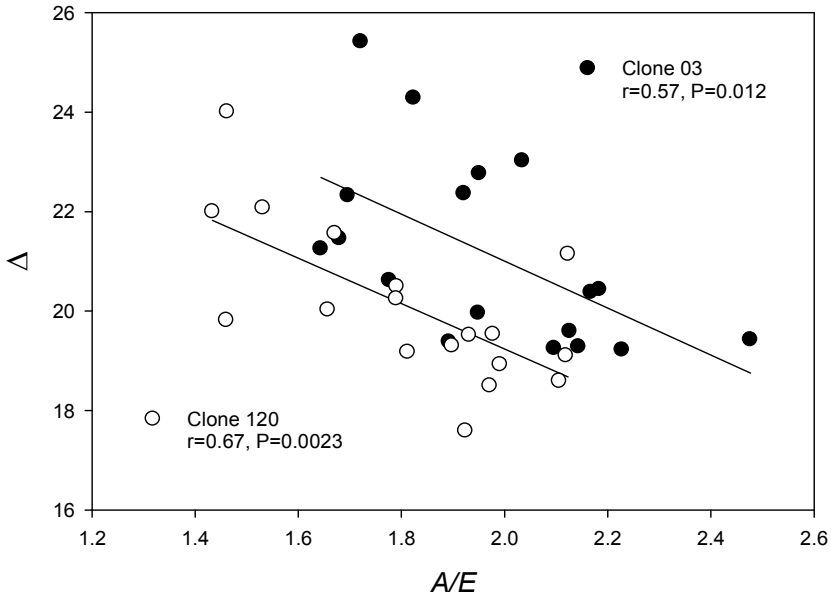

Fig. S1. The relationship between instantaneous water use efficiency and carbon isotope discrimination in two clones of robusta coffee.

GENERAL CONCLUSIONS

Our predictions that the physiological performance and biomass accumulation of plants depend on the total amount of light received and on the temporal scales of diurnal light availability were confirmed in the experiments that were conducted with *C. arabica* seedlings. The higher biomass accumulation increased in association with morphogenetic processes that were induced by higher light availability during the morning (faster leaf area formation) and ultimately resulted in better photosynthetic productivity on the whole-plant level. Exposing plants to light during the morning promoted an increased *A* and higher carbohydrate availability in the long term, which led to improved growth with no major alterations in biomass allocation patterns; this could be a consequence of ontogenetic drift in coffee plants. Photosynthetic rate changes during the day were associated with stomatal limitations and were apparently unrelated to carbohydrate accumulation or photoinhibition within the varying light regimes. In summary, growth and physiological performance depends not only on the total amount of PAR received by the plant per day but also on the temporal order of diurnal variations in PAR supply.

In the second experiment, in contrast to our working hypothesis, it was shown that *C. canephora* clones grown under harsh environmental conditions and that were shaded during the morning displayed better gas exchange performance throughout the day, and there were varying abilities to cope with PAR alterations between the clones under evaluation. It was also confirmed that the acclimation of Robusta coffee to temporal changes in PAR were more associated with morphological and physiological traits, particularly those related to light capture. In addition, *C. canephora* clones were shown to have a robust antioxidative system that allows adequate photoprotection in response to the total amount of PAR received, which was observed by the absence of cellular damage and photoinhibition. Furthermore, we showed that morning shade may increase the resource use efficiencies with minimal changes in CC, MC and leaf chemical composition under temporal variations of light availability. Overall, the present data indicates that this species has considerable phenotypic plasticity, which may be used to select promising genotypes for intercropping with shelter trees to improve light use efficiency when PAR is limiting and could lead to an increased carbon gain, as found in clone 03.

Finally, the results obtained in both experiments are of great practical importance and should be considered for the management of light availability as an alternative to the current cultural management of coffee plantations in response to climate change scenarios, particularly in mountainous areas or in marginal environments where better growth (and production) is a major goal.