Ministério da Ciência, Tecnologia e Inovação - MCTI

Instituto Nacional de Pesquisas da Amazônia - INPA

Programa de Pós-Graduação em Ciências de Florestas Tropicais - PPG-CFT

GROWTH, PHOTOSYNTHETIC RESPONSES AND ACCLIMATON OF TREE SPECIES TO DIFFERENT LIGHT ENVIRONMENTS

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MANAUS, AMAZONAS

FEVEREIRO, 2019

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Tese apresentada ao Programa de Pós-Graduação em Ciências de Florestas Tropicais do Instituto Nacional de Pesquisas da Amazônia (MCTI-INPA) como parte dos requisitos para a obtenção do título de Doutor em Ciências de Florestas Tropicais.

MANAUS, AMAZONAS

FEVEREIRO, 2019

Nina Junior, Adamir da Rocha

Growth, photosynthetic responses and acclimation of tree species to different light environments / Adamir da Rocha Nina Junior --- Manaus: [s.n],2019

122 f:il

Tese (Doutorado) --- INPA, Manaus, 2019.
Orientador: José Francisco de Carvalho Gonçalves
Área de concentração: Ciência de Florestas Tropicais

1. Grupos sucessionais. 2. Espécies arbóreas. 3. Fisiologia do estresse. 4. Fotossíntese. I. Título

SINOPSE:

As respostas fotossintéticas e a aclimatação de plantas jovens de seis espécies arbóreas tropicais de grupos sucessionais distintos em diferentes condições de irradiância foram investigadas. Características funcionais foliares foram relacionadas as taxas de crescimento e as principais limitações à fotossíntese, a partição do nitrogênio foliar, o desempenho fotoquímico e o sistema de defesa antioxidante em resposta a irradiância foram determinados utilizando-se análises multivariadas.

Palavras-chave: Estresse, espécies arbóreas, fotossíntese e fotoinibição.

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA, INOVAÇÕES E COMUNICAÇÕES

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DE FLORESTAS TROPICAIS

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Ata da Defesa Pública da Tese de Doutorado de **ADAMIR DA ROCHA NINA JÚNIOR,** aluno (a) do Programa de Pós-Graduação *Stricto Sensu* em CIÊNCIAS DE FLORESTAS TROPICAIS - CFT, realizada no dia 28 de fevereiro de 2019.

Aos vinte e oito dias do mês de fevereiro de 2019, às 09h00, na Sala de Aula I do PPG-CFT, Campus III, INPA-V8, realizou-se a Defesa Pública da Tese de Doutorado intitulada: **"GROWTH, PHOTOSYNTHETIC RESPONSES AND ACLIMATING OF TROPICAL SPECIES TO CONTRASTING LIGHT ENVIRONMENTS"**, em conformidade com o Artigo 68 do Regimento Interno do PPG-CFT e Artigo 52 do Regimento Geral da Pós-Graduação do Instituto Nacional de Pesquisas da Amazônia (MCTI-INPA) como parte final de seu trabalho para a obtenção do título de **DOUTOR (A) EM CIÊNCIAS DE FLORESTAS TROPICAIS.** A Banca Examinadora foi constituída pelos seguintes professores doutores: **FLÁVIA CAMILA SCHIMPL (IFAM), JOÃO HENRIQUE FROTA CAVALCANTI (UFAM), VINÍCIUS FERNANDES DE SOUZA (UEA), NIWTON LEAL FILHO (INPA) e RONALDO RIBEIRO DE MORAIS (EMBRAPA).** O (a) Presidente da Banca Examinadora, Dr. José Francisco de Carvalho Gonçalves, deu início à sessão convidando os senhores membros e o (a) doutorando (a) a tomarem seus lugares e informou sobre os procedimentos a serem observados para o prosseguimento do exame. A palavra foi, então, facultada ao(à) Doutorando(a) que apresentou uma síntese do seu estudo e respondeu às perguntas formuladas pelos membros da Banca Examinadora. Depois da apresentação e arguição, a referida Banca Examinadora se reuniu e decidiu por

aprovar por unanimidade.

A sessão foi encerrada às 13h e, para constar eu, Ana Serra Campos, Secretária do PPG-CFT lavrei a presente Ata, que depois de lida e aprovada foi assinada pelo Presidente e membros da Banca Examinadora.

Banca Examinadora:

Dr (a) FLÁVIA CAMILA SCHIMPL	[X] Aprovado(a)	[ ] Reprovado(a)	
Dr (a) JOÃO HENRIQUE F. CAVALCANTI	[X] Aprovado(a)	[ ] Reprovado(a)	
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Dr. José Francisco de Carvalho Gonçalves
Presidente da Banca/Orientador

Dr. Niro Higuchi
Coordenador do PPG-CFT
PO. N° 242/2017

DEDICATÓRIA

Aos meus pais, Adamir e Maria Ney da Rocha Nina,

Por todo amor, incentivo, dedicação e exemplo.

À minha esposa Natasha e minha filha Luísa,

Pelo amor, compreensão, carinho e apoio incondicional.

AGRADECIMENTOS

Ao Instituto Nacional de Pesquisas da Amazônia - INPA e ao Programa de Pós-graduação em Ciências de Florestas Tropicais (CFT) pela oportunidade de cursar o doutorado.

Ao Instituto Federal de Educação, Ciência e Tecnologia do Amazonas – IFAM, pela concessão da licença que me permitiu finalizar a tese.

À CAPES pela concessão da bolsa de estudos.

Ao corpo docente do CFT pelos ensinamentos proporcionados durante o mestrado e o doutorado.

Ao meu orientador, Dr. José Francisco de Carvalho Gonçalves, pela atenção, confiança, incentivo e orientação no desenvolvimento não somente da tese, mas de toda minha vida acadêmica. Obrigado pelos bons exemplos e pela oportunidade de trabalhar, crescer e aprender ao seu lado.

Aos Drs. Jair Maia e Samuel Martins, meus coorientadores, pelas valiosas contribuições ao trabalho. Foi inspirador conviver com pessoas tão dispostas a ajudar e ensinar.

Ao Dr. Wagner Araújo (UCP-UFV) e sua equipe, em especial a Dra. Auxiliadora Martins, pelo acolhimento em Viçosa e apoio em algumas análises bioquímicas.

Aos meus pais, Adamir da Rocha Nina e Maria Ney da Rocha Nina, pelo amor e carinho que me deram e pelo incentivo para continuar os estudos.

Ao meu irmão Bruno da Rocha Nina e a minha irmã Adriana Nina Cáceres Toro, pela convivência e pelo apoio durante toda a vida mesmo quando geograficamente separados.

À minha amada esposa Natasha pelo amor, cumplicidade, apoio e incentivo em todos os momentos. Só você sabe quão difícil foi chegar até aqui e eu tenho certeza que se fosse preciso faríamos tudo de novo, juntos!! Melhor companheira para a vida Deus não poderia ter me concedido!

À minha filha Luísa, luz da minha vida, sua existência é minha maior alegria e motivação para seguir em frente.

À equipe do Laboratório de Fisiologia e Bioquímica Vegetal (LFBV-INPA), em especial Josiane e Rafaela, por toda ajuda ao longo do trabalho e pelos momentos de amizade e descontração que compartilhamos.

Aos amigos que passaram pelo Laboratório e contribuíram para meu crescimento profissional desde o início desta jornada (Ulysses, Ronaldo, Renata, Carlos e Glaudecy).

Meus mais sinceros agradecimentos!

ABSTRACT

Light is a fundamental resource for energy flow in biological systems and is highly determinant for plant life. The great variability in the natural environment requires that plants, especially trees, exhibit a great ability to adjust metabolism to spatial and seasonal variations in the availability of this resource, aiming not only to maximize the capture and use of light, but also the development of efficient mechanisms in the dissipation of excess light energy to avoid photoinhibitory damages to photosynthetic apparatus. In this context, the objective of this study was to evaluate the photosynthetic plasticity and light acclimation capacity of six Amazonian tree species (H*ymenea courbaril, Carapa guianensis, Hevea brasiliensis, Tabebuia serratifolia, Bellucia grossularioides* and *Ochroma pyramidale*) divided into different successional groups (two late successionals, two mid-successional and two pioneers, respectively) in response to different light conditions (full sunlight [FS - 100 % of solar radiation, which simulates a clearing area], moderate shade [MS - provide by shade cloths reducing direct incident solar radiation] and deep shade [DS - provided by adult tree canopies, which simulate an understory light environment]).The photosynthetic circadian rhythm and NSC accumulation/turnover, relative growth and biomass accumulation rates, maximum photosynthesis and their limitations, leaf N partition, antioxidant system activity and transients of chlorophyll *a* fluorescence were evaluated in four individuals per species and per environment using multivariate analyzes. Regardless of the light regime, the limitations to daily photosynthetic course were preponderantly diffusive. The pioneer species and *H. courbaril* exhibited the highest photosynthetic rates and higher accumulation of biomass in FS. In DS, greater photosynthesis was observed for *B. grossularioides*, however, this did not reflect in a greater accumulation of biomass while in this environment. The morphological changes most correlated to acclimation were leaf area in DS and leaf gain in FS. The leaf nitrogen partition between photosynthetic and structural compounds was more determinant for photosynthesis than its own content, since the ability to modulate the N allocation is fundamental for increasing or decreasing photosynthesis as a consequence of changes in the light environment. There was higher N foliar investment in Rubisco by the pioneers in FS, which also showed higher photosynthetic rates, electron transport and carboxylation rate in this environment. Despite the smaller V_{cmax} in DS, the plants exhibited a higher activation state of Rubisco suggesting a better ability to take advantage of sunflecks, especially in late successional species. Regarding the antixodative system, the SOD activity was fundamental for the reduction of cell damage in FS, but this activity decreased n lower irradiances. Only the late successional *C. guianensis* exhibited photoinhibition in FS and the pioneer *O. pyramidale* did not survive on DS, suggesting that acclimation at high irradiance is less challenging than intense shading. The results suggest that growth while in a shaded environment is apparently determined more by factors related to starch accumulation and turnover than photosynthesis. Additionally, it can be concluded that the plasticity to regulate the leaf's physiological and morphological adjustments and the capacity of acclimation in response to changes in light environment regime is not necessarily related to the successional group of species.

RESUMO

A luz é um recurso primordial para o fluxo de energia nos sistemas biológicos sendo determinante para a vida das plantas. A grande variabilidade com que se apresenta em ambiente natural exige das plantas, em especial de árvores, que exibam grande capacidade de ajustar o metabolismo a variações espaciais e sazonais quanto a disponibilidade deste recurso visando não apenas a maximização na captura e utilização de luz, mas também o desenvolvimento de mecanismos eficiente na dissipação do excesso energia luminosa para evitar danos fotoinibitórios ao aparato fotossintético. Diante deste contexto, este estudo objetivou avaliar a plasticidade fotossintética e a capacidade de aclimatação de seis espécies arbóreas da Amazônia (*Hymenea courbaril*, *Carapa guianensis*, *Hevea brasiliensis*, *Tabebuia serratifolia*, *Bellucia grossularioides* e *Ochroma pyramidale*) pertencentes a grupos sucessionais distintos (duas sucessionais tardias, duas intermediárias e duas pioneiras , respectivamente) em reposta a diferentes condições de luminosidade (pleno sol[FS – 100% da radiação solar, simulando ambientes abertos]; sombreamento moderado[MS–35% de FS, providenciado pelo uso de sombrites] - e sombreamento intenso[DS – 5% de FS, sob a copa de árvores adultas, simulando ambiente de sub-bosque]). Foram avaliados o ritmo circadiano fotossintético e o acúmulo/turnover de CNE, as taxas de crescimento relativo e acúmulo de biomassa, a fotossíntese máxima e suas limitações, partição do N foliar, atividade do sistema antioxidante e as características da fluorescência da clorofila *a*. Independente do regime de luz as limitações ao curso diário fotossintético foram preponderantemente difusivas. As espécies pioneiras e *H. courbaril* exibiram as maiores taxas fotossintéticas e maior acúmulo de biomassa em FS. Em DS *B. grossularioides* exibiu maior fotossíntese, mas isso não refletiu em acúmulo de biomassa. As alterações morfológicas mais relacionadas a aclimatação foram a área foliar em DS e o ganho de folhas em FS. A partição do nitrogênio foliar entre compostos fotossintéticos e estruturais foi mais determinante para a fotossíntese do que seu próprio conteúdo, sendo a capacidade de modular a alocação do N fundamental para o aumento ou diminuição da fotossíntese em função de alterações no ambiente de luz. Houve maior investimento de N foliar em Rubisco pelas plantas pioneiras em FS, que exibiram também maiores taxas fotossintéticas, transporte de elétrons e velocidade de carboxilação neste ambiente. Apesar do menor V_{cmax} em DS, as plantas exibiram maior estado de ativação da Rubisco sugerindo melhor capacidade de aproveitamento de sunflecks, especialmente pelas sucessionais tardias. Quanto ao sistema antioxidativo, a atividade da SOD foi fundamental para redução de danos celulares em FS, tendo menor participação com a diminuição da irradiância. Apenas a sucessional tardia *C. guianensis* exibiu fotoinibição em FS e a pioneira *O. pyramidale* não sobreviveu em DS, sugerindo que a aclimatação a alta irradiância é menos desafiadora do que o sombreamento intenso. Os resultados demonstraram que crescimento a sombra aparentemente é mais determinado por fatores relacionados ao acúmulo e turnover de amido do que da fotossíntese. Adicionalmente, pode-se concluir que a plasticidade para regular os ajustes fisiológicos e morfológicos na folha e a capacidade de aclimatação em resposta às mudanças no regime de luz do ambiente não estão necessariamente relacionadas ao grupo sucessional da espécie.

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LIST OF ABREVIANTIONS

A	Measured photosynthesis
A_n	Net photosynthesis rate
A_{max}	Maximum photosynthesis rate
APX	Ascorbate peroxidase
BL	Biochemical limitation
CAT	Catalase
C_c	Carbon dioxide concentration at the site of Rubisco carboxylation
C_i	Sub-stomatal carbon dioxide concentration
DS	Deep shade environment
E	Transpiration rate
ETR	Electrons transport rate
ETR_C	Electrons transport rate to carboxylation
ETR_O	Electrons transport rate to oxygenation
FS	Full sunlight environment
F_v/F_m	Maximum quantum yield of PSII
F_v'/F_m'	Maximum efficiency of PSII photochemistry in the light
g_m	Mesophyll conductance
g_s	Stomatal conductance
IS	Photosynthetic induction state
IWUE	Intrinsic water efficiency use
J_{max}	Maximum potential electron transport rate
LA	Leaf area
LCP	Light compensation point
LGI	Leaf gain index
LMA	Leaf mass area
LMF	Leaf mass fraction
LSP	Light saturation point
ML	Mesophyll limitation
MS	Moderate-shade environment
NAR	Net assimilatory rate
NPQ	Non-photochemical quenching
NSC	Non-structural carbohydrates
PAR	Photosynthetically active radiation
P_b	N fraction in electron transport chain proteins
PCA	Principal component analysis
P_l	N fractions in pigments involved in the light capture
ϕNO	Quantum yield of non-regulated energy dissipation in PSII
ϕNPQ	Quantum yield for dissipation by down-regulation (thermic energy)

POX	Polyphenol peroxidase
PPFD	Photosynthetic photon flux density
ϕPSII	Effective quantum yield of PSII
P_r	N fraction in Rubisco
P_s	N fraction in structural components
qL	Photochemical quenching by “lake-model”
R_d	Dark-respiration
RGR-BIO	Relative growth rate in biomass
RGR-D	Relative growth rate in diameter
RGR-H	Relative growth rate in height
RGR-LA	Relative growth rate in leaf area
RGR-LM	Relative growth rate in leaf mass
RGR-RM	Relative growth rate in root mass
RGR-SM	Relative growth rate in steam mass
RMF	Root mass fraction
ROS	Reactive oxygen species
R_P	Photorespiration rate
SL	Stomatal limitation
SLA	Specific leaf area
SMF	Steam mass fraction
SOD	Superoxide dismutase
T*	CO_2 compensation point in the absence of mitochondrial respiration in the light
TBARS	Thiobarbituric acid reactive substance
Vc_{max}	Maximum Rubisco carboxylation velocity
WUE	Water efficiency use

1. GENERAL INTRODUCTION

It is a current fact in scientific literature that tropical forests are important for the maintenance of not only regional, but also global climate. In this context, the Amazon forest stands out as having a large carbon stock in its biomass; half the total terrestrial biomass, and housing a quarter of the global biodiversity (Lee et al., 2013). All these characteristics have developed under the damp equatorial climate in which varied conditions of light, temperature, water and edaphic factors represent challenges to plant establishment.

Among the abiotic factors, light is primordial for the energy flow in biological systems, as it is highly determinant for physiological processes of plants. In the forest environment, it is possible to observe a great heterogeneity regarding the availability of light both in terms of the spatial aspect (canopy, understory or clearing areas) and temporal character (seasonality). In addition to this heterogeneity, anthropic action and/or natural events can promote frequent and significant changes in apparently balanced environments, making the establishment of a species and its continuity in the ecosystem is related to ability to adjust quickly its metabolism to light conditions provided by the environment, that is, its phenotypic plasticity and acclimation capacity (Morais et al., 2007; Li et al., 2008; Baird et al., 2017;Marenco et al., 2017).

Acclimation can be defined as an increasing of plant tolerance to stress due previous exposure to stressful conditions of one or more resources necessary for the proper functioning of plant metabolism (Vialet-Chabrand et al., 2017). Regarding light stress, some ecological models involving the plasticity of tropical forest species have established that the flexibility of photosynthetic response of a species is related to the pattern of variability of the environmental conditions in the species naturally occurring habitat, that is, its photosynthetic plasticity and capacity for acclimation are closely linked to the successional group to which it belongs. According to these models, shade-tolerant species, that generally colonize the understory, would have less flexibility of response, on the other hand, pioneer or light-demanding species, subject to more heterogeneous and unstable conditions, would exhibit more plastic responses (Bazzaz and Picket, 1980; Chadzon et al., 1996; Valladares and Niinemets, 2008). However, increasing evidence indicates that both light demanding and shade tolerant species are able to exhibit high phenotypic plasticity, which suggests that the flexibility of adjustments in response to new environmental conditions is not necessarily related to the successional status of the species, but rather to its greater or less individual ability to modulate the photosynthetic apparatus and, consequently, changes in the carbon metabolism (Rozendaal et al., 2006; DosAnjos et al., 2015).

This process of light acclimation involves a complex set of physiological, biochemical and structural adjustments with the objective of increasing the capture and use of light within the limits of the genetic constitution of the plant. When exposed to changes in the irradiance conditions, the plants respond with a modification in functional foliar characteristics, which represent one of the most important aspects to be considered during the acclimation process (Niinemets et al., 2015;

Valladares et al., 2016). However, it still hasn't been clearly elucidated if, how and why leaf functional traits differ significantly among these ecological groups.

Functional traits related to leaf morphology and anatomy tend to respond to environmental changes and influence physiological functions (Poorter et al., 2009; Zhang et al., 2017). Among them, variation in the ratio between mass and leaf area (LMA-leaf mass area) has been widely used as an indicator of phenotypic plasticity, growth and photosynthetic potential of plants under contrasting conditions of irradiance, given LMA's important significance for leaf economy spectrum (LES), which describes a set of trade-offs among characteristics related to the carbon balance in plants, in a ratio between the efficiency of acquisition and allocation of the primary resources available in the environment (Edwards et al., 2014; Reich, 2014; Onoda et al., 2017). Frequently, increase in LMA has been associated with higher mesophyll conductance (g_m), which would reduce CO_2 restrictions at the carboxylation site, and, thus increase photosynthesis (Niinemets and Tenhunen, 1997; Flexas et al., 2012; Peguero-pina et al., 2015).

In addition to morphological aspects, acclimation also involves metabolic processes and must be understood from the biochemical view, especially regarding changes in the context of thylakoid proteins, pigments and enzymes which are regulated by signals that are stimulated by environmental events occurring around the leaf. This denotes the importance of considering the relationships between the allocation of organic and inorganic compounds and the content of the mineral nutrients in the leaves (Moon et al., 2015; Vialet-Chabrand et al., 2017; Kalaji et al., 2018).

All these modifications occur in order to maximize the capture and use of light and to minimize the occurrence of photoinhibition. The term photoinhibition describes a reversible or irreversible decline of photosynthetic activity when light is absorbed beyond the photosynthetic capacity of the plant. It is a state of physiological stress that can occur in all photosynthetic organisms, and tree species in particular are largely subject to fluctuations in the light regime to which they will be subject throughout their life cycle (Dietz, 2015; Lestari and Nichols, 2017).

One way to reduce the effects of intense radiation is to dissipate excess energy in non-photochemical processes, such as heat dissipation via the xanthophyll cycle, and in the form of fluorescence. In addition, to avoid oxidative damage, plants have enzymatic and non-enzymatic systems for ROS (reactive oxygen species) removal which may be directly related to the tolerance of plants to a stress situation (Dietz, 2015; Retkute et al., 2015).

Faced with all these dynamics of the luminous regime, understanding the mechanisms of plasticity, which can maximize or limit the ability and the photosynthetic performance in varied environments, is therefore one of the main objectives of the physiological investigations in plants (Baird et al., 2017). In light of the above, this work sought to understand the strategies of acclimation to light stress in species from different successional groups submitted to different conditions of exposure to irradiance, both daily and long term by answering the following questions:

1. Does the growth of saplings obey the same patterns under limiting conditions of irradiance? Are they defined by succession groups or respond individually and specifically under limited conditions of irradiance?

2. What functional leaf traits are most strongly associated with the success of acclimation to different light environments? Are they the same between species and environments?

3. Are the traits that drive the acclimation process to high irradiance the same as those that can enhance the performance under low light?

This thesis was separated into three chapters. In the first, we studied the circadian photosynthetic rhythm and accumulation/turnover of non-structural carbohydrates (NSC), as well as their implications for the growth of plants in different environments. In the second, 34 leaf functional traits and their effects on the photosynthetic performance were evaluated. In the third, the effects of irradiance on the light harvest complex, the photochemical yield, the functioning of the antioxidant system and possible photoinhibitory damages were determined.

2. OBJECTIVES

2.1 GENERAL OBJECTIVE:

The main objective of this thesis was to investigate the functional traits of six tree species growing under different light environments (high, moderate and low irradiance) in order to understand the ecophysiological responses and identify functional adaptations that drive and justify the success of acclimation to environments with different light.

2.2 SPECIFIC OBJECTIVES:

1. Determine the changes in the relative growth rates and the biomass partition influenced by the light environment (Chapter 1).

2. To investigate the circadian rhythm of photosynthesis and the daily turnover of non-structural carbohydrates (Chapter 1).

3. Study the photosynthetic performance in response to irradiance (maximum photosynthesis, respiration in the dark, irradiance compensation and saturation) (Chapter 2).

4. Investigate the photosynthetic limitations in the process of acclimation to high and low irradiance and the partition of leaf N between the photosynthetic structures (Chapter 2).

5. Investigate the strategies used by plants for increasing the efficiency of capture and dissipation of excess light energy (Chapter 3).

6. To study the photochemical and antioxidative metabolism performance to prevent or reduce cell damage as a function of irradiance (Chapter 3)

7. Evaluate the common characteristics or contrasting strategies among species and successional groups studies for acclimation to different light environments (Chapter 1, 2 and 3).

CHAPTER 1

GROWTH AND DAILY COURSE OF CARBON BALANCE OF SAPLINGS OF TREE SPECIES SUBJECTED TO DIFFERENT LIGHT ENVIRONMENTS

GROWTH AND DAILY COURSE OF CARBON BALANCE OF SAPLINGS OF TREE SPECIES SUBJECTED TO DIFFERENT LIGHT ENVIRONMENTS

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ABSTRACT

Light is a highly heterogeneous environmental factor that influences the growth and survival of plants. More than a source of energy for photosynthesis, light is a signal used to trigger growth and structural differentiation between plants. In order to study the effect of light availability on the growth of plants of different succession groups, six tree species were submitted to three different light environments (full sunlight-FS, moderate shade-MS and deep shade-DS).The photosynthetic circadian rhythm, the daily course of accumulation and turnover of non-structural carbohydrates (NSC), the relative growth rates and functional (stomatal conductance, chlorophyll fluorescence transients and N and P content) and morphological (mass and area) leaf traits that may be associated with biomass growth (RGR-BIO) were evaluated. Pioneer species exhibited higher net photosynthesis (A_n), electron transport rates (ETR) in FS, followed by a late successional species (*H. courbaril*). In DS, one of the pioneers did not survive the shade treatment *(O. pyramidale*) and the other one again had the highest A_n. In general, there was a higher turnover of starch in the FS plants, where A_n was also associated with RGR-BIO. In DS, these correlations were not observed, indicating significant changes in the relation between NSC stock, turnover and growth. Under full sunlight the growth of the pioneers was favored, however, under shading, late successional were more efficient, even with lower photosynthetic rates. The most significant leaf traits for RGR-BIO increases among the environments also differed, with leaf gain index (LGI) being more significant in FS, whereas in DS the leaf area expansion (RGR-LA) was more important. In general, the reduction of photosynthesis during the day is regulated by diffusive limitations which are influenced by stomatal closure, without signs of metabolic retroinhibition or imbalance in the source-sink relationship. Under favorable conditions of irradiance, there was a direct relationship between photosynthesis and growth. However, under deprivation of the resource, this relationship was probably altered due to differences in the allocation and turnover of NSC, especially starch and sucrose.

Key-words: Photosynthesis, acclimation, non-structural carbohydrates and biomass.

Chapter formatted according to *Environmental and Experimental Botany* journal instructions

1. INTRODUCTION

Light is a highly heterogeneous environmental factor that influences plant growth and development. In addition to being a source of energy for photosynthesis, light is a signal used to trigger growth and structural differentiation between plants, thus, when subjected to variations in the quality and intensity of irradiance, most of them are capable, at a higher or lesser extent, to acclimatize themselves to the changes (Valladares et al., 2016).

The ability of a genotype to express variation in adaptive characteristics in response to environmental changes is known as phenotypic plasticity. Since irradiance is one of the environmental factors that is most critical to the growth and establishment of plants, those with higher plasticity are more capable of surviving in heterogeneous environments and/or under stressful conditions related to this factor (Valladares, 2008; Gonçalves et al., 2012; Gaburro et al., 2015).

Some studies indicate that the tolerance range of plants to different light intensities varies according to ontogenetic stages and successional groups. Studies involving acclimation of saplings of tree species of different ecological groups to full sunlight environment provide reports of more pronounced photoinhibition in late successional species, which are adapted to shade conditions and exhibit more conservative strategies for slow growth in the understory. On the other hand, to pioneer or light-demanding species, that exhibit acquisitive strategies for rapid growth, the efficiency in dissipating excess energy ensures the maintenance and even the enhancement of photosynthetic performance under full sun conditions (Gonçalves et al., 2005; Favaretto et al., 2011; Azevedo and Marenco, 2012).

However, increasing evidence indicate that both light demanding and shade tolerant species are capable of exhibiting phenotypic plasticity, suggesting that the flexibility of adjustments in response to new environmental conditions is not necessarily related to the successional status of the species and its greater or less modular capacity to photosynthetic machinery (Rozendaal et al., 2006; DosAnjos et al., 2015)

These adjustments of the photosynthetic process are modified according to the intensity of the irradiance, in this way, in forest environments the photosynthetic apparatus must be able to use the incident light efficiently, since the light availability varies due to the great heterogeneity of the environment. In addition to spatial heterogeneity, it is important to highlight that light variations during the day can also affect the photosynthetic process and, consequently, the synthesis and the allocation of photoassimilates, with implications for plant growth (Dias and Marenco, 2006; Thalmann and Santelia, 2017).

Growth rates vary greatly among plant species, especially when subjected to different light stimulus (Evans and Poorter, 2001; Sterck et al., 2013). Leaf functional traits play an

important role not only in plant acclimation, but also play a key role in enhancing carbon gain. The most studied leaf traits in the acclimation process are related to morphological changes (e.g. specific leaf area) and/or to physiological processes (e.g. respiration, net photosynthesis, carboxylation rate and nutrient use efficiency) resulting from an increase or decrease in irradiance (Wright et al., 2004; Sánchez-Gómez et al., 2006; Martínez-Garza et al., 2013; Niinemets et al., 2017; Guimarães et al., 2018). However, the metabolism of sugars generated in the photosynthetic process can also be considered a functional process in acclimation since disturbances in the process of synthesis and degradation of these metabolites can cause strong impacts on plant growth(Stitt and Zeeman, 2012).

Studies relating the synthesis, daily accumulation and turnover of photoassimilates to growth are scarce in woody plants, most of the carbon allocation models were constructed based on herbaceous plants (Gibon et al., 2009; Pilkington et al., 2015). However, in plants in general, starch and sucrose are the main products of photosynthesis. The sucrose synthesized during the day is exported to supply the demands of sink organs (root, stem and young leaves) and the starch is stored in the leaves to be degraded at night and provide carbon for maintenance and growth demands of plant. Therefore, since light affects the regulatory mechanisms of synthesis and degradation of starch, knowing its effects on different functional groups of plants in different environments can contribute to the understanding of the growth mechanisms of tree species.

In this study, we aimed to study the growth of six tree species of three distinct successional groups in different irradiance environments based on the daily course of carbon and the accumulated biomass allocation to answer the following questions: 1) Does the growth of species vary according to the successional group in which they are classified? 2) Does the maintenance of high photosynthetic rates promote higher growth rates irrespective of functional and successional groups or environmental conditions? 3) Can the largest accumulation of photoassimilates in high irradiance environments lead to a reduction of the photosynthetic rates throughout the day and can the opposite favor the maintenance of greater photosynthesis in shaded plants?

2. MATERIAL AND METHODS

2.1 Plant material and growth conditions

The study was conducted at the National Research Institute for the Amazon - INPA (Manaus, Amazonas - Brazil). Saplings of 6 native Amazonian species belonging to three distinct succession groups (Table 1) were cultivated in the nursery and when they reached 9 months of age were transplanted to plastic pots containing12 liters of substrate (regional latosoil collected in the native forest with organic matter – see supplementary material for nutritional characteristics). At 12-14 months of age, one part of the group was transferred to 2 different irradiance environments and one part was kept in the nursery (4 individuals for specie in each treatment). Incident photosynthetic radiation was monitored with a line quantum sensor (model LI-191, LI-COR Inc., Lincoln, Nebraska, USA) for 7 sunny days (see appendix for details).

Table 1: List of studied species with scientific name, family and successional group as described in literature.

Specie	Family	Sucessional group	Reference
Hymenea courbaril	Fabaceae	Late-sucessional	Souza et al. (2009)
Carapa guianensis	Meliaceae	Late-sucessional	Vinson et al. (2005)
Hevea brasiliensis	Euphorbiaceae	Mid-sucessional	Amaral et al. (2009)
Tabebuia serratifolia	Bignoniaceae	Mid-sucessional	Gualberto et al. (2014)
Bellucia grossularioides	Melastomataceae	Pionner	Bentos et al. (2017)
Ochroma pyramidale	Malvaceae	Pionner	Slot and Winter (2018)

The light treatments (Table 2) consisted of full sunlight (FS) (100 % of solar radiation, simulating a forest clearing), artificial moderate shade (MS) provided by shade cloths reducing direct incident solar radiation (simulating an understory light environment with partial canopy openness) and natural deep shade (DS) with natural shadow provided by adult tree canopies (simulating an understory light environment). The plants were subjected to these treatments during 180 days.

Table 2: Daily PAR average, maximum PAR observed, average percentage of full sunlight under three light conditions.

Specie	Full Sunlight (FS)	Moderate Shade (MS)	Deep Shade (DS)
PAR average	1027.51±10.49	362.50 ± 9.37	47.95 ± 7.98
Maximum PAR	1866.17 (13:00h)	795.61 (12:00h)	74.50 (14:00h)
% PAR	100	35.23	4.66

2.2 Growth analysis

The determination of plant growth variables (height, collection diameter, leaf area and number of leaves) were performed at the beginning and at the end of the experiment (180 days later). The height and diameter were measured using a digital caliper. Leaf area (LA) was measured using a leaf area meter (CID Inc., Camas, WA, USA). For the determination of the masses, the plants were sectioned in leaf, aerial part and root, and later conditioned in a forced ventilation oven at 65ºC until reaching mass constant.

The relative growth rates in height (RGR-H), diameter (RGR-D), leaf area (RGR-LA), leaf gain index (LGI), steam mass (RGR-SM), root mass (RGR-RM), leaf mass (RGR-LM) and net assimilation rate (NAR) were calculated according to Hunt (1990) and Davanso et al. (2002) following the equations:

$$RGR = (\ln X_f - \ln X_i)/(t_f - t_i) \quad \text{Equation 1}$$

$$LGI = (NL/OL)*[100 / (t_f - t_i)] \quad \text{Equation 2}$$

$$NAR = (PM_f - PM_i) / (t_f - t_i) * (\ln LA_f - \ln LA_i) / (LA_f - LA_i) \quad \text{Equation 3}$$

Where in equation 1 X_f and X_i represent final and initial values for desired variable, in equation 2 NL = new leaves and OL = original leaves and in equation 3 PM = plant mass and LA=leaf area. For all equations t_f and t_i represent final and initial time of experiment.

Specific leaf area was calculated by the ratio between leaf area and dry leaf mass obtained from leaf discs with known area (Evans and Poorter, 2001).

2.3 Biomass allocation

The biomass allocation, which consists of the leaf mass fraction (LMF), stem mass fraction (SMF), and RMF, was determined as follows (Poorter et al., 2012):

LMF = leaf biomass/total plant biomass Equation 4

SMF = stem biomass/total plant biomass Equation 5

RMF = root biomass/total plant biomass Equation 6

2.4 Leaf gas exchange, photosynthetic and fluorescence circadian rhythm

Leaf gas exchange was measured with a portable open gas exchange system (LI-6400, LI-COR Biosciences Inc., Lincoln, Nebraska, USA) equipped with a blue/red light source (LI-6400-02B, LI-COR). The net photosynthetic rate (A_n), dark respiration (R_d), transpiration rate (E), intracellular carbon (C_i) and stomatal conductance (g_s) were measured in mature and full expanded leaves of each sapling in three moments on the same day (7h-9h, 11h-13h and 15h-

17h). The photosynthetic radiation was standardized from previous measurements performed during the day in each environment. The fixed parameters were the CO_2 flux (400 μmol s^{-1}), temperature (30 °C) and relative humidity around 60%.

The same leaves were subjected to actinic light (1500 μmol m^{-2} s^{-1}) for 60s followed by the application of a pulse of saturating light (8000 μmol m^{-2} s^{-1} during 0.8s). The quantum yield of photosystem II (ϕPSII) and the rate of electron transport (ETR) was calculated by using the formulas of Maxwell and Johnson (2000).

2.5 Determination of leaf of glucose, fructose, sucrose and starch contents

The foliar contents of soluble sugars and starch were determined by the enzymatic method (Fernie et al., 2001). Leaves samples were collected at 06:00, 12h:00, 18h:00 and 06h:00 again (the day after) and immediately frozen in liquid nitrogen and stored at -80°C until biochemical assays were performed. Lyophilized foliar samples were subjected to ethanol extraction and the content of glucose, fructose and sucrose were determined in ethanol-soluble fraction. The starch fraction was determined from the insoluble fraction.

2.6 N and P content

The leaves samples were dried in an oven at 65 °C to mass constant. The total N was determined by the Kjeldahl method. P was extracted by digestion with 3:1 nitric-perchloric solution and was determined by spectrophotometry at 725 nm (Vitti and Ferreira, 1997)

2.7 Experimental design and statistical analysis

The experimental design was factorial and completely randomized (6X3) with six species in three treatments. After the assumptions of normality and homoscedasticity were complied, in order to analyze the differences between species and treatments, the appropriate analyses of variance and averages were performed using the Tukey post hoc test. Where appropriate, relationships between variables were tested by regression equations, using, as criteria for adjustment: 1) significance of the adjusted regression; 2) significance of its coefficients and 3) higher coefficient of determination.

All analyses were performed with SPSS (IBM SPSS Statistics 23) and Sigma Plot 11 (Systat software, 2008).

3. RESULTS

3.1 Growth and biomass allocation

Pioneer species were higher in RGR-D when in FS than in all other conditions (Figure 1A). All plants showed lower RDR-D in DS, with *T. serratifolia* showing lower performance in this environment, but, conversely, presented highest RGR-H among all species and treatments (Figure 1B). Contrasting with RGR-D, *O. pyramidale* obtained lower RGR-H in FS, accompanied by *B. grossularioides*, *H. brasiliensis* and *C. guianensis,* which exhibited the lowest rates in FS and MS. Only *H. courbaril* and *T. serratifolia* were lower in RGR-H in DS than FS and MS (2.5 and 2 times, respectively).

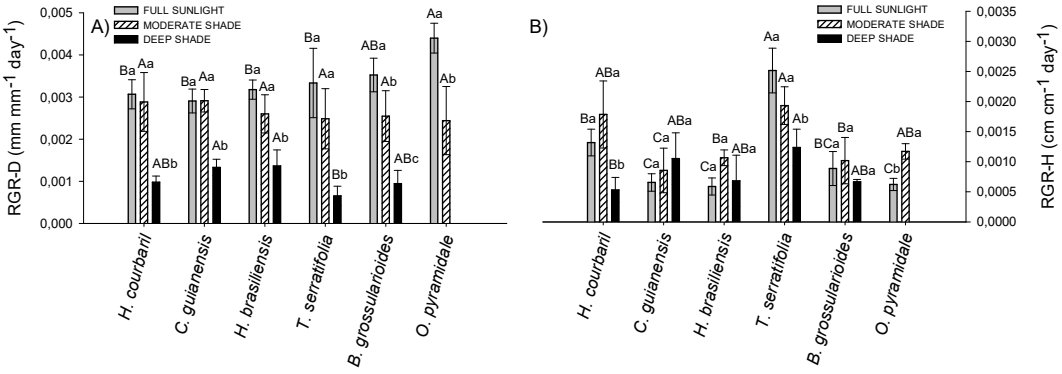

Figure 1: Relative growth rate in A) diameter (RGR-D) and B) height (RGR-H) of saplings of six tree species submitted to three different light environments. Same capital letters for different species in same environment and lower case for same species in different environment are equal by Tukey test ($p<0.05$).

Regarding biomass relative growth, all plants obtained the lowest rates in DS for LM, SM, RM and, consequently, for total biomass (Figure 2A, C, E, G and H). *H. courbaril* was the species which exhibited the highest RGR-BIO in all environments, along with the pioneers *O. pyramidale* and *B. grossularioides* in FS.

The negative rates in RGR-LM and RM for all species in DS, except *H. courbaril* should be noted. For RGR-BIO in DS, only *H. courbaril* and *C. guianensis* showed growth in this environment. All others suffered from a proportional decrease in relation to initial biomass.

The patterns of biomass allocation revealed different strategies among the different environments. In FS and MS, most plants exhibited higher investments in roots (RMF) than another compartments, only *C. guianensis* made a proportional investment between root and aboveground part (Figure 2B, D and F). *T. serratifolia* showed higher RMF than all others, but, as expected, plants in DS showed higher LMF and SMF.

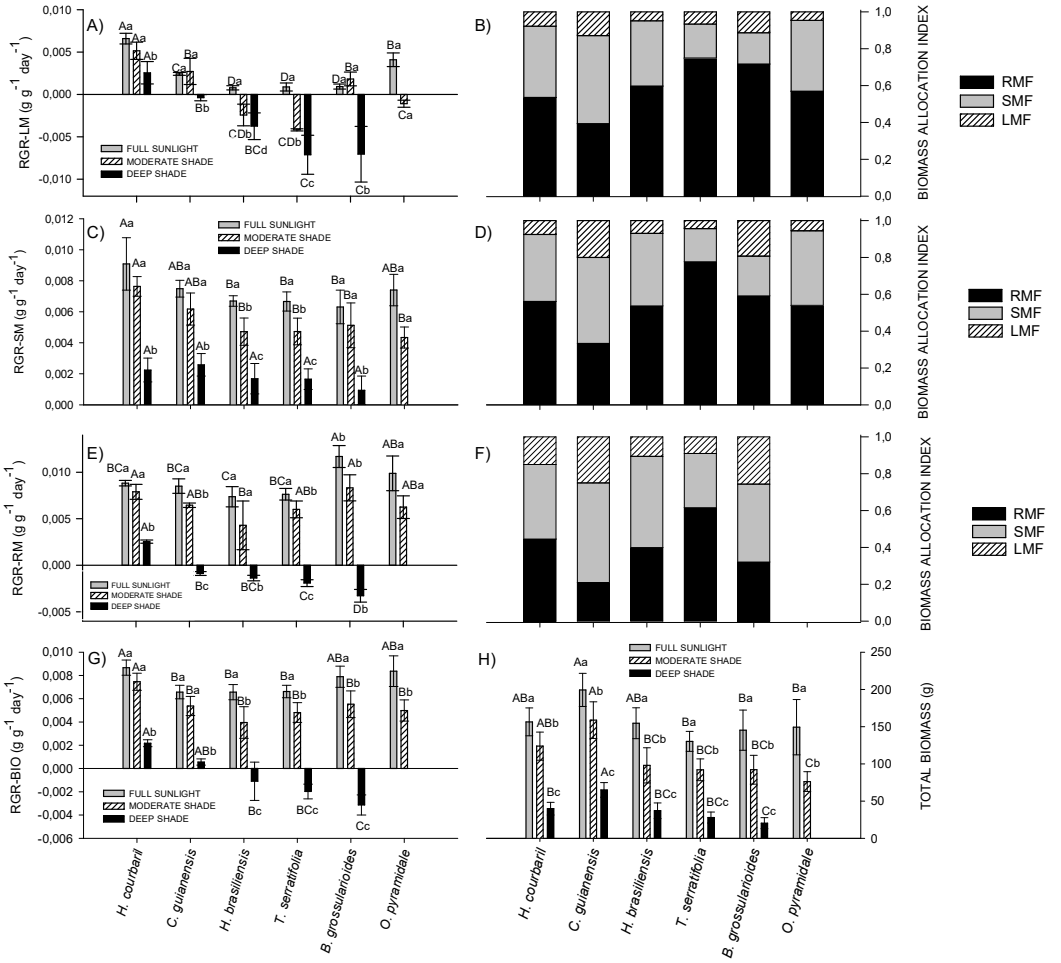

Figure 2: Relative growth rate in A) leaf mass (RGR-LM), C) stem mass (RGR-SM), E) root mass (RGR-RM), G) plant biomass (RGR-BIO), H) total biomass and biomass allocation index of saplings of six tree species submitted to three different light environments (B-full sunlight, D-moderate shade and F-deep shade). Same capital letters for different species in same environment and lower case for same species in different environment are equal by Tukey test ($p<0.05$).

Analyzing morphological and photosynthetic traits and its implications for biomass growth (Figure 3), we found positive effects of RGR-LA on RGR-BIO in MS and DS ($p=0.0009$, $R^2=0.402$ and $p<0.0001$, $R^2=0.774$), but this did not affect FS, which was influenced by IGF ($p=0.0007$, $R^2=0.415$). In general, RGR-BIO was related to NAR ($p<0.001$, $R^2=0.652$), SLA ($p<0.001$, $R^2=0.652$), N ($p<0.001$, $R^2=0.496$) and A_n ($p<0.001$, $R^2=0.524$).

Effects on A_n were observed for IGF ($p=0.0131$, $R^2=0.250$) and P in FS ($p=0.0003$, $R^2=0.449$). NAR ($p<0.001$, $R^2=0.504$), SLA ($p<0.001$, $R^2=0.504$), N ($p<0.001$, $R^2=0.155$) were related in MS.

P was weakly correlated with RGR-LA in FS ($p=0.0311$, $R^2=0.194$) as N ($p<0.001$, $R^2=0.207$), which showed correlation with NAR ($p<0.001$, $R^2=0.265$) and SLA in FS ($p=0.0036$, $R^2=0.326$) and DS ($p<0.001$, $R^2=0.659$).

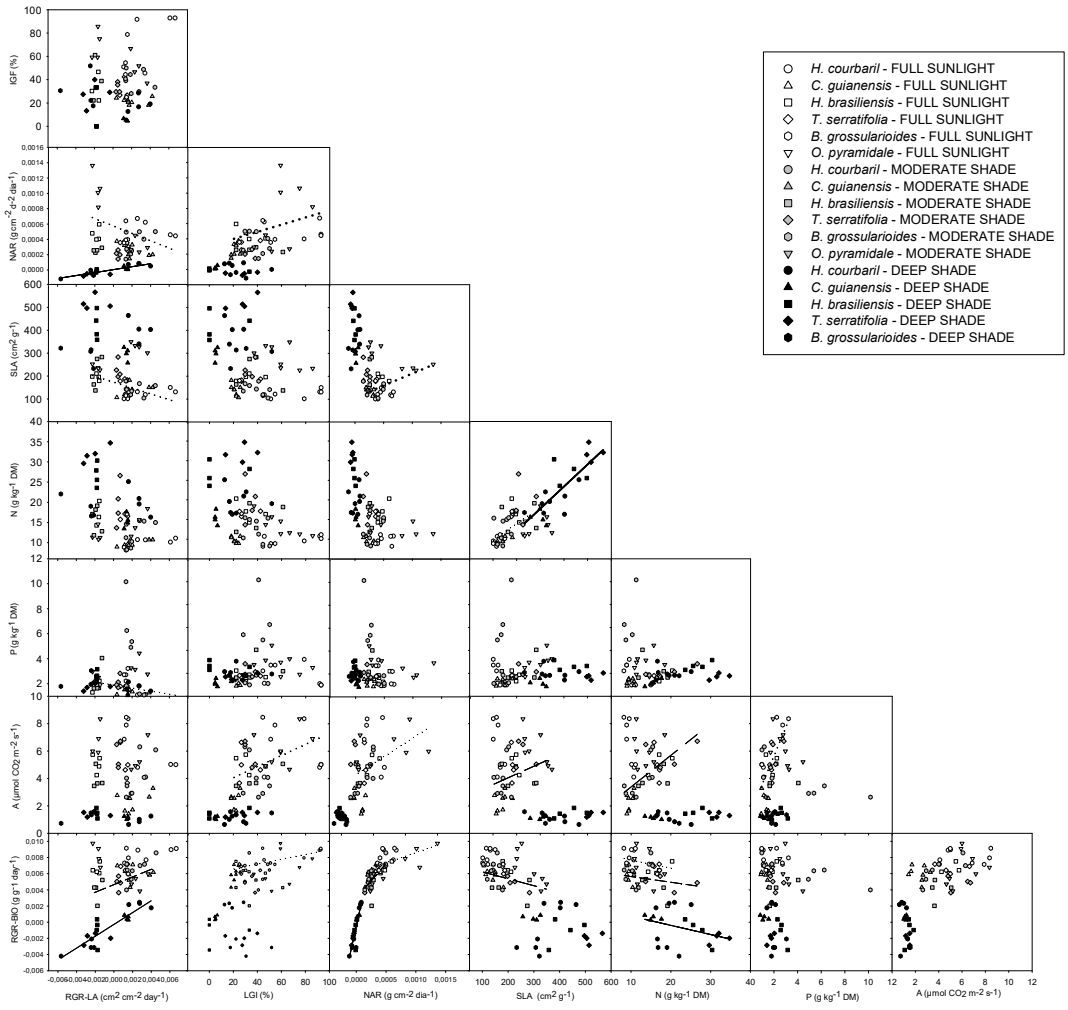

Figure 3: Linear regressions between growth variables and biomass accumulation in six tree species submitted to three light environments. Lines indicate significant regressions.

3.2 Photosynthetic circadian rhythm

Considering the differences between the species in each environment, *H. courbaril* and *C. guianensis* (both late successionals), as well as *T. serratifolia* (intermediary) all presented

higher net photosynthesis (A_n) in MS (7, 3.9 and 8.2 µmol CO_2 $m^{-2}s^{-1}$, respectively) between 11:00-13:00 when compared to other times and environments (Figure 4A). For *H. brasiliensis*, the highest observed value was also at this time, however in FS (6.4 µmol CO_2 $m^{-2}s^{-1}$). The pioneers, *B. grossularioides* and *O. pyramidale,* displayed better photosynthetic performance between 07:00-13:00 in FS (10.8 and 8.8 µmol CO_2 $m^{-2}s^{-1}$, respectively). The first, however, suffered drastic reductions (close to 80%) at other times and in other environments, while *O. pyramidale* underwent a lower reduction under these conditions and was able to exhibit similar A_n in MS between 11:00-13:00. However, despite more consistent performance than *B. grossularioides* between FS and MS, all individuals of *O. pyramidale* in DS were already dead after 97 days of the experiment.

In FS environment, of the six species studied, *B. grossularioides* and *O. pyramidale*, both pioneers, exhibited the highest daily averages of photosynthesis and the highest net photosynthetic rates (A_n) between 07:00 and 13:00, accompanied by the highest values of stomatal conductance (g_s) (Figure 4 A and B).These species exhibited their highest A_n during the first few hours of the day, maintaining high rates until 13:00h, from whence they showed a marked decrease of 79.8% and 41.9%, respectively, observed from 15:00. With the exception of *C. guianensis* (64%), all other non-pioneer species showed a lower reduction of the A_n at the end of the day compared to the first measurement in relation to the pioneer species.

Contrary to FS, where small differences occurred between morning and midday, in the MS environment, the highest A_n results were displayed between 11:00 and 13:00. *H. courbaril* and *O. pyramidale* exhibited net photosynthesis 2.7 and 2.1 times higher than at 7:00-9:00, respectively. For *C. guianensis* and *T.serratifolia* and for *H. brasiliensis* and *B. grossularioides* the values were approximately 1.8 and 1.5 times larger, in this same sequence.

For the DS environment, all values observed for the six species studied were lower than those observed in the higher irradiance environments for the entire day (0.3 –2.7 µmol CO_2 $m^{-2}s^{-1}$). Similar to that of the plants in MS, the highest A_n were exhibited between 11:00. and13:00 (1.5 – 2.7 µmol CO_2 $m^{-2}s^{-1}$). However, the decrease in photosynthesis in this environment was higher from 15:00 than in other environments, reaching approximately 50% for *H. courbaril* (0.8µmol CO_2 $m^{-2}s^{-1}$) and *C. guianensis* (0.9µmol CO_2 $m^{-2}s^{-1}$), both late successionals, and 67% for *H. brasiliensis* (intermediate). The other intermediate species, *T. serratifolia*, showed the largest reduction, of 88%, followed by the pioneer *B. grossularioides* with 84% (0.31 and 0.49 µmol CO_2 $m^{-2}s^{-1}$). All individuals of *O. pyramidale* (pioneer) were already dead after 97 days of the experiment.

In all cases, for all species and environments, there was a reduction of A_n throughout the day, especially for *B. grossularioides* and *O. pyramidale*, apparently associated with diffusive limitations for photosynthesis, as shown by the correlation between A_n and g_s and of the diurnal decreases of C_i (Figure 4C-D).

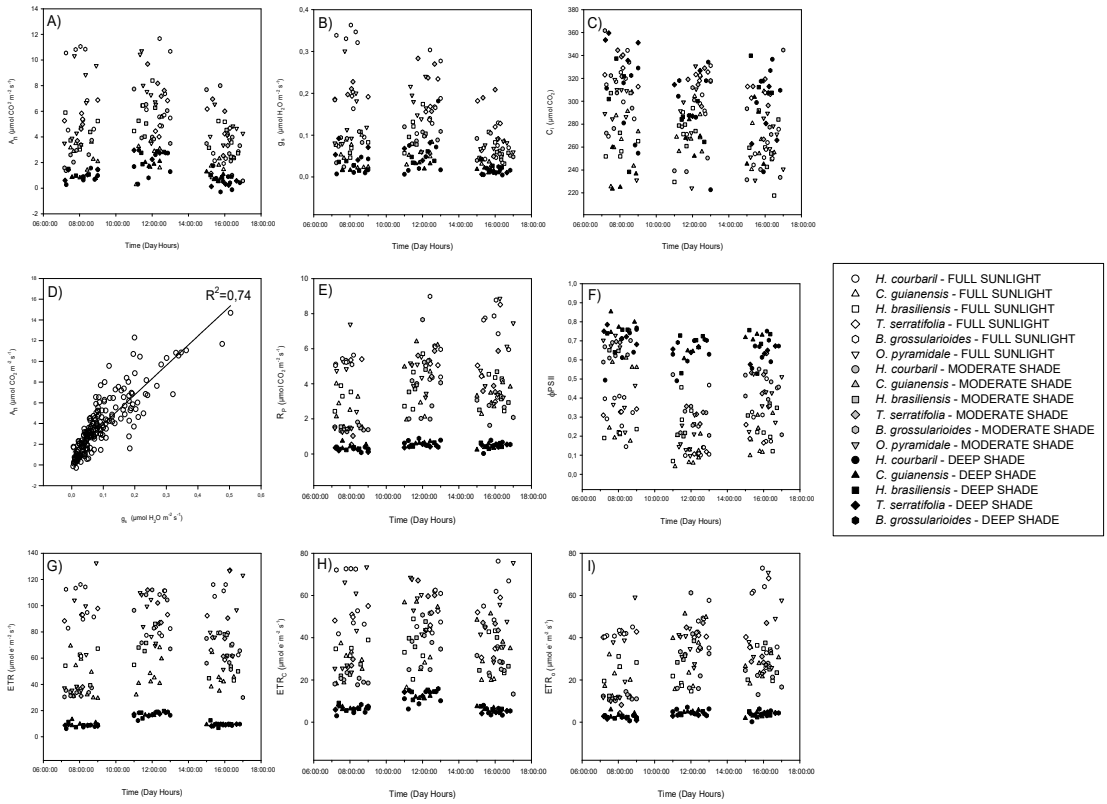

Figure 4: Circadian rhythm of photosynthetic parameters: A) net photosynthesis (A_n), B) Stomatal conductance (g_s), C) Intracellular CO_2 (C_i), D) Correlation A_n/g_s, E) Photorespiration rate (R_P), F) Quantum yield of photosystem II (ϕPSII), G) Total electron transport rate (ETR), H) Fraction of electron used for carboxylation (ETR_C) and I) Electron flow cost for photorespiration (ETR_O) in saplings of six tree species submitted to different light environments.

The photorespiratory rates (R_P) were higher in FS in the early morning (3.3 – 5.3 µmol CO_2 $m^{-2}s^{-1}$) and at the end of the day (3.4 – 8.9 µmol CO_2 $m^{-2}s^{-1}$), with the latter being noticeably higher in the pioneer species. Between 11:00 and 13:00, there was an increase in MS, which decreased at dusk. Throughout the day, R_P was very low in DS (0.2 – 0.7 µmol CO_2 $m^{-2}s^{-1}$) (Figure 4E).

The quantum yield of photosystem II (ϕPSII) was lower for plants in FS after 09:00(0.10-0.35) than another environment (016-0.52 and 0.60-0.72 for MS and DS, respectively) (Figure 4F). During the day, with an increase in irradiance, ϕPSII decreased in all environments, but this was more remarkable in FS and MS (between 30%-70%). In general, plants in DS showed constant values all the time with lower decreases at 11:00 (5%-20%).

All non-pioneers showed the highest electron transport rates (ETR) between 11:00-13:00 in MS (48-102 µmol e^- $m^{-2}s^{-1}$) (Figure 4G). The pioneer species obtained higher ETR in FS, *O. pyramidale* at 15:00(121.51 µmol e^- $m^{-2}s^{-1}$) and *B. grossularioides* 11:00 (197.5 µmol e^- $m^{-2}s^{-1}$). In

MS, there was a tendency to increase ETR from 11:00 and decrease it from 15:00 onwards. Apparently, variations in ETR were driven by the change in irradiance during the day affecting the balance between the flow of electrons destined for carboxylation (ETR_O) and the photorespiratory cost (ETR_C) (Figure 4H-E). All species showed lower ETR in DS (7.5-17.9 μmol e^- $m^{-2}s^{-1}$), however, although much smaller than that observed in other environments, between 11:00 and-13:00, values were almost twice the rates observed at the beginning and the end of the day.

The highest photochemical performance of pioneer species in FS was notable. But, with irradiance decreases among environments, the differences between pioneers and species from other successional groups also decreased.

3.3 Daily course of metabolites

In general, the highest glucose contents were observed in the intermediate species *H. brasiliensi*s and *T. serratifolia*, with the first showing higher values than all the others (Figure 5 A1-A3). Although the values were higher, there was a low daily amplitude of this metabolite in this species, the highest variation was observed for *H. courbaril* in FS (102%) and *B. grossularioides* in FS and DS (41% and 98%, respectively).

A small daily amplitude was observed in fructose contents in most species in all environments (Figure 5 B1-B3). The exceptions were *T. serratifolia* in FS and MS (140%) and *B. grossularioides* in DS (72%).The variations in sucrose and starch were higher in FS and MS than in DS, with the emphasis on *H. courbaril*, *T. serratifolia*, B*. grossularioides* and *O. pyramidale* (Figures 5 C1-D3).

The starch content in FS was higher than in all the soluble sugars in FS and MS environments, only *H. brasiliensis* and *T. serratifolia* presented similar sucrose and glucose contents of starch in these environments. Contrarily to FS and MS environments, there was more accumulation of soluble sugars than starch in DS, and *H. courbaril* and *C. guianensis* accumulated more sucrose and other species accumulated more glucose.

Glucose, sucrose and starch turnover were, in most cases, higher in FS than in other environments. For fructose, the largest turnover was in MS. It should be noted that B*. grossularioides* exhibited a higher turnover of soluble sugars than the other species in DS, even surpassing the values observed for this same species in other environments.

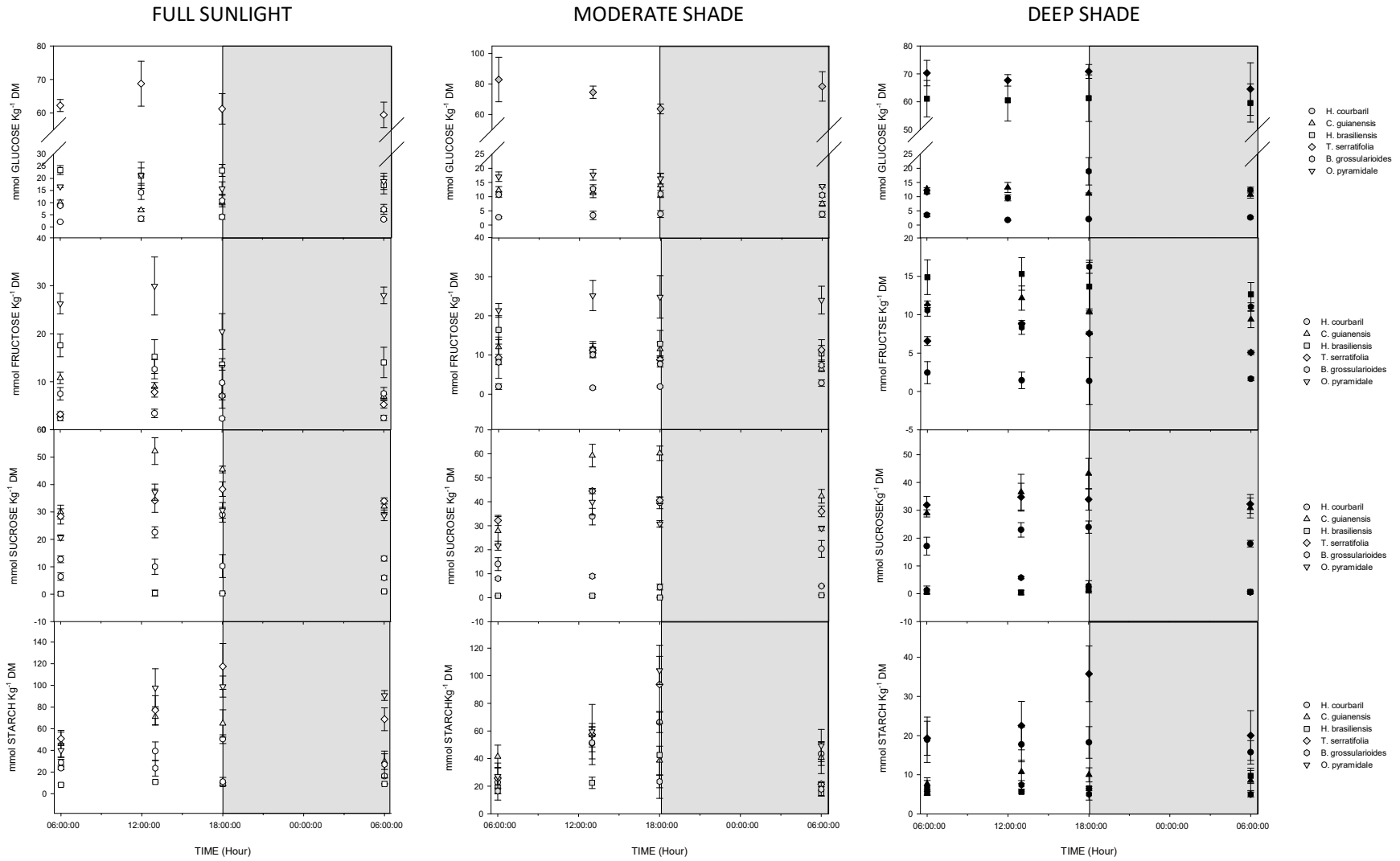

Figure 5: Leaf contents of (A) glucose, (B) fructose, (C) sucrose and starch in saplings of six tree species submitted to three light environments: full sunlight (open symbols), moderate shade (gray symbols) and deep shade (black symbols). Values are means (n=4) and vertical bars represents standard error.

4. DISCUSSION

4.1 Growth and biomass allocation

As expected, there was a higher RGR-D in the saplings in environments with higher irradiance (FS and MS) for all species when compared to DS. Similar results were found for many tropical tree species, including some of the species studied (Souza and Válio, 2003; Portes et al., 2010; Santos et al., 2014; Cunha et al., 2016). RGR-H was also lower in DS plants than in other environments, however, despite the lower absolute value, the ratio between height and diameter growth was higher in DS, which is a characteristic common to trees that grow under shading (Valladares, 2008).

For growth in total biomass (RGR-BIO) in FS, there was no difference between pioneers and the late successional *H. courbaril*, which exhibited even greater growth in MS and DS, suggesting that there is no close relationship between growth and this ecological group conventionally described in literature, although pioneer species have accumulated lower biomass in DS. Saplings in FS also exhibited higher biomass in all compartments compared to DS plants. It is worth noting that the intense shading condition imposed difficulties for the growth of renewable tissues such as leaves and roots, causing negative values for RGR-LM and RGR-RM for most DS species. In these plants, there was a greater investment in stem mass (RGR-SM), which may be a consequence of the greater proportional increase in height in the search for light or even shade avoidance (Poorter et al., 2012; Keenan and Niinemets, 2016).

These proportional differences between compartments reveal a lot from the point of view of light modulated biomass allocation responses. Plants exposed to full sunlight almost always exhibit greater transpiration flows and demand large and efficient root systems, therefore they need robust vascular systems, which in a way can favor the greater growth in root and stem mass in this environment, justifying higher RMF and SMF in MS and FS. On the other hand, in DS, under milder microclimates, in terms of leaf temperature and lower transpiration flow, there was greater allocation of carbon above ground to the detriment of the roots which aimed at an increase in light capture surface (Gonçalves et al., 2012; Zhang et al., 2013).

These growth responses are partly the product of the morphophysiological changes influenced by the light environment in order to increase the efficiency in the absorption and the use of this resource (Gibert et al., 2016). Leaf morphological characteristics related to light interception, such as RGR-LA and IGF exert an effect on growth rates and biomass accumulation (Toledo-Aceves et al., 2017). Under light scarcity, the growth is slower and leaf area expansion (greater RGR-LA) is an important strategy in order to increase the interception of light energy, on the other hand, under high irradiance the reduction of leaf surface represents a decrease in the water loss due to transpiration and the continuous gain of new leaves (greater IGF) replaces structures eventually damaged by excess energy from new leaves and adapted (morphologically and biochemically) to the new light condition. In regards the allocation of the resources in the leaf, as observed by Guimarães et al.(2018) there was no clear

pattern between SLA and growth associated with successional groups, but, unlike in Li et al. (2017) there was a positive correlation between SLA and RGR-BIO in this study.

The NAR exhibited correlation with RGR-BIO in all environments, and was strongest in DS. This correlation was also expected between A_n, RGR-LA and IGF since this variable reflects the proportion of the assimilator system that is involved in dry mass production, and may present positive or negative values depending on the conditions to which the plant is submitted (Concenço et al., 2015). Higher growth rates generally have a positive relationship with leaf nutrient contents, mainly N and P. However, in this study the relationship with growth occurred only with N (Rüger et al., 2012; Li et al., 2017).

4.2 Photosynthetic circadian rhythm

Late successional species under high irradiance conditions generally exhibit more pronounced photoinhibition and lower photosynthetic performance when compared to pioneer species (Lestari and Nichols, 2017). On the other hand, in more shaded environments, such as forest understory, a better photosynthetic performance of this successional group is expected. However, although pioneer species require a high amount of light to supply the highest energy cost of maintaining their metabolism, some of them demonstrate the ability to modulate photosynthesis when cultivated under low irradiance, exhibiting performance similar to that of late successionals in these environments (Portsmuth and Niinemets, 2007; Gonçalves et al., 2012; DosAnjos et al., 2015).

Under high irradiance, the highest net photosynthesis rate of the pioneer species was notable in comparison to non-pioneer species. On the other hand, while *O. pyramidale* did not survive the low availability of light energy in DS, *B. grossularioides* exhibited superior performance when compared to late successionals in the early hours of the day, demonstrating high photosynthetic plasticity for shade tolerance.

Shade tolerance is defined as the ability to maximize light capture and carbon gain efficiency under conditions of low light availability (Valladares, 2008). However, if shade tolerance is related to higher carbon gain, it would be expected that, under low light, shade tolerant species would exhibit higher photosynthetic rates than intolerant species. In this study, this was partially true, since one pioneer species exhibited a photosynthetic performance in the shade superior to that of the late successional ones and another pioneer did not survive in the shaded environment. These results corroborate the suggestion that shade tolerance is not only associated with maximization of light capture and its use in photosynthetic process, but with the ability to maintain captured resources and the conservative use of resources (Reich, 2014; Valladares et al., 2016).

In any case, the reductions in A_n observed for all species were associated to proportional reductions in g_s and C_i, indicating diffusive limitations as the potential cause of the decrease of the photosynthetic activity during the day. The more pronounced reduction of g_s in FS indicates the need for stomatal closure in order to reduce water loss due to transpiration (higher temperature associated

with high irradiance) in this environment. In DS, on the other hand, the maintenance of the stomatal opening for a longer period of time aimed to improve the use of daytime light and promoting an increase in CO_2 assimilation (Allen and Pearcy, 2000). However, lower g_s are observed at the end of the day in all environments, which suggests strong control of endogenous factors in a circadian rhythm (Dodd et al., 2005).The involvement of an endogenous clock in the modulation of the stomatal movement would be of benefit to the plant, since keeping the stomata closed at night (when carbon absorption is zero) avoids unnecessary water loss by transpiration.

The R_P responded in a contrary manner to the ϕPSII. The lower R_P rates in MS and DS led to lower proportional reductions for photosynthesis and for ϕPSII in these environments. The highest ETR were observed for the pioneers in FS, sustaining the high photosynthetic rates exhibited. However, the greater electron flows for non-assimilative processes (ETR_O) evidences the increase of R_P in this environment and reinforces the photoprotective role of oxygenation for the photosynthetic machinery under high irradiance during the day when there is CO_2 limitation to the Calvin cycle (Voss et al., 2013; Way et al., 2015), in this case imposed by the reductions in g_s and consequently in C_i.

4.3 Daily course of metabolites

Studies on the daily course of carbon are scarce among tree plants, however, all indicate starch as an important growth regulator which plays an important physiological role with the emphasis on energy supply in periods of absence of light (night) or low irradiance (Zeeman et al., 2005; Thalmann and Santelia, 2017).

In this study, the highest content of starch at the end of the day was found in environments with higher irradiance. With the exception of *H. brasiliensis* and *B. grossularioides*, plants in FS maintained between 50% and 60% of the total non-structural carbohydrates (NSC) accumulated as starch at dawn. On the other hand, in DS only *H. courbaril* kept approximately 50% of NSC stocked in this way, while the other species maintained only between 10-20%.Carbon allocation models based on herbaceous plants characterize this residual starch as nonproductively sequestered carbon, since it is not available to the nocturnal metabolism and, consequently, its incomplete remobilization would limit growth rates (Stitt and Zeeman, 2012). It should be noted, however, that the validity of the extrapolation of these carbon allocation models for tree species is uncertain (Dietze et al., 2014) and since, in this study. species with higher amounts of residual starch exhibited higher growth in biomass than those with lower residual starch in all environments, it is clear one must not generalize with regard to these models and the importance of further studies on tree species for the construction of a carbon allocation model based on physiological controls appropriate to structural differences among plant groups.

Allied to structural differences, plants are also sensitive to environmental modifications, so that expression of carbohydrate-regulated genes may influence acclimation processes, mainly related to the mechanisms of sugar allocation (Dietze et al., 2014; Thalmann and Santelia, 2017). Unlike starch, the soluble sugars differed slightly between FS and DS. The exception was *H. brasiliensis*, which

exhibited a higher content of glucose than other sugars at all times, raising the proportion between soluble sugars among the NSC.

The maintenance of high levels of soluble sugars plays an important role in the osmoregulation and stomatal control of plants under high irradiance, favoring the absorption of water by reducing the cellular water potential and reducing the loss of water in conditions of excessive transpiration. In addition, defense against oxidative damage may be involved since they act in signaling pathways of reactive oxygen species (Ende and Valluru, 2009). In the shaded environment, with a tendency to reduce carbohydrate contents due to the lower photosynthetic rates, the reserve of any type of carbohydrate is important in order to supply the costs of maintaining the metabolism during conditions of scarcity or even light deprivation (Myers and Kitajima, 2007)but the larger synthesis of soluble sugars responds more quickly to energy costs during the day. Considering that starch is the main source of carbohydrate used for growth, the low accumulation of this photoassimilate at the end of the day associated to high with a high turnover may have been the limiting factor for the growth of the pioneer species *B. grossularioides* in DS. This result reinforces the importance of conservative strategies in regards to the use of carbon to increase the shade tolerance and could explain, at least in part, the low growth and high mortality of pioneer species under shaded conditions even if they present higher photosynthetic rates than late successionals.

Considering the effect of the accumulation of carbohydrates on the gas exchanges, metabolic retroinhibition can not be indicated as a direct cause of the reduction of A_n during the day because photosynthesis varied regardless of the sucrose and starch contents, reinforcing the evidence for diffusive limitations as the main factor for the decrease in A_n.

5 CONCLUSION

Species with higher photosynthetic rates present a higher accumulation of biomass under high irradiance; however, this does not apply to shaded environments and does not obey the expectations for successional groups. The morphophysiological alterations associated with leaf characteristics contribute in a differentiated way to acclimation and biomass accumulation between light environments, foliar gain (LGI) prevails for high irradiance and growth in leaf area (RGR-LA) for low irradiance. In this way the use of acquisitive strategies was more efficient in environments with higher light availability, while conservative strategies and the accumulation of NSC can determine not only the growth, but the survival of plants when under restriction of the light resource.

The reduction of photosynthesis along the day was influenced by diffusive limitations imposed by stomatal closure, with few or no metabolic retroinhibition associated with accumulation of photosynthesis final products, regardless of the successional group or the growth strategy of the species.

Finally, studying the mechanisms involved in the accumulation of NSC after turnover, especially residual starch, may contribute to understanding the mechanisms involved in the growth of tree species under low irradiance.

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CHAPTER 2

PHOTOSYNTHETIC PLASTICITY OF TREE SPECIES DURING ACCLIMATION TO DIFFERENT LIGHT ENVIRONMENTS

PHOTOSYNTHETIC PLASTICITY OF TREE SPECIES DURING ACCLIMATION TO DIFFERENT LIGHT ENVIRONMENTS

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ABSTRACT

Among the abiotic factors that play key role on the physiological processes, light stands out as the primary energy flux in biological systems, and is highly determinant for plant growth and development. However, the heterogeneous form it presents in nature requires that the plants exhibit great ability in regards to spatial and seasonal changes in the availability of this resource. Frequently the greater or lesser flexibility of plants responses is associated to the successional group where the species are inserted. In order to study the variations in the photosynthetic plasticity to different light environments, saplings of six tree species were submitted to three different environments of luminosity (full sunlight-FS, moderate shade-MS and deep shade-DS).The maximum photosynthesis (A_{max}), leaf N partition, stomatal, mesophilic and biochemical limitations (SL, ML and BL, respectively), carboxylation velocity (V_{cmax}) and electron transport (J_{max}) rates and the state of induction photosynthetic (IS) were evaluated. Higher values of A_{max}, V_{cmax} and J_{max} in FS were recorded for pioneer species, which invested the largest leaf N in Rubisco. However, in DS *O. pyramidale* did not withstand shading. There was no variation in A_{max} for *H. brasiliensis* and *C. guianensis*, which showed little flexibility in the partitioning of leaf N between the environments. The IS of plants in DS was higher than those of FS, especially for late successionals. The lower IS for pioneers reveals its lesser ability for taking advantage of *sunflecks*. In general, the main limitations are diffusive, with SL and ML having equal importance in full sunlight and ML decreasing along with the irradiance given effect of LMA on g_m. The variables that direct the photosynthetic process respond independently in relation to the successional group, especially when in low irradiance. The efficient partitioning of leaf N between photosynthetic and structural components was shown to be determinant for the acclimation process and increase or decrease of photosynthesis among the environments.

Key-words: Acclimation, photosynthetic responses, N partition and Rubisco activation state.

1. INTRODUCTION

Light provides the energy required to photosynthesis, therefore, it determines the distribution, survival, growth and development of plants. Thus, plants, especially trees, might be able to respond positively to variations in light availability in their natural environment, given the great heterogeneity with which light presents itself. In a complex canopy, for example, light availability can vary up to 200 times between the canopy and the lower strata of forest(Li et al., 2008; Marenco et al., 2017). In addition to the natural heterogeneity, the light factor has been indicated as the main cause for the decline in distribution of some rare or endangered species, where the loss or fragmentation of habitat due to deforestation can lead to microclimatic changes, and light environments can become significantly different from the original, creating stress situations for plants (Aleric and Kirkman, 2005; Liu et al., 2006).

To deal with this heterogeneity, plants have the capacity for considerable phenotypic plasticity, in other words, changes in a set of functional attributes that comprise morphological and physiological characteristics in order to adapt to new conditions and thereby use more efficiently the primary resources with the aim of increasing their photosynthetic rates (Gratani, 2014; Baird et al., 2017).

Some ecological models involving the plasticity of tropical forest species have established that the flexibility of photosynthetic response of a species is related to the pattern of environmental condition variability in their natural habitat, that is, their photosynthetic plasticity and acclimation capacity is closely linked to the successional group to which they belong. According to these models, shade-tolerant species, that generally colonize the understory, would present less flexibility of response and pioneering or light-demanding species, subject to more heterogeneous and unstable conditions, would present more plastic responses (Bazzaz and Picket, 1980; Chadzon et al., 1996). However, increasing evidence indicates that both light demanding and shade tolerant species are capable of exhibiting phenotypic plasticity, which suggests that the flexibility of adjustments in response to new environmental conditions is not necessarily related to the successional status of the species and its greater or lesser modular capacity to photosynthetic process (Rozendaal et al., 2006; DosAnjos et al., 2015). In view of these divergences, understanding the mechanisms of plasticity through which plants maximize fitness in varied environments is, therefore, one of the main objectives of physiological investigations in plants (Baird et al., 2017).

Anatomical and functional traits related to leaf morphology tend to respond to changes in environmental variables and thus, influence physiological function. Traits as changes in LMA (leaf mass area) have been commonly used to assess differences among species, but may also be relevant for the understanding of intra-specific responses as a result of contrasting light conditions (Poorter et al., 2009).

The higher photosynthetic capacity has been related to higher LMA, which is often associated with higher mesophyll conductance (g_m) (Niinemets and Tenhunen, 1997; Flexas et al., 2012) which would reduce CO_2 restrictions at the carboxylation site, and increase photosynthesis. Other studies suggest that changes in photosynthesis under different light conditions are dominated largely by the level and reallocation of leaf nitrogen (Frak et al., 2001; Moon et al., 2015). These variations, however, are determined not only by inherent species characteristics, but also by the plant growth environment.

In this context, the objective of this study was to investigate the photosynthetic plasticity and acclimation capacity of six native Amazonian tree species in response to different light environments. For that, 34 variables involved in the photosynthetic process were studied in order to answer the following questions: 1) is the photosynthetic plasticity of acclimation irradiance related to successional groups? 2) What are the decisive characteristics for the success of the photosynthetic acclimation process and what are the major limitations? 3) Are they the same among different species and environments?

2. MATERIAL AND METHODS

2.1 Plant material and growth conditions

The study was carry out at the National Institute for Amazonian Research - INPA (Manaus, Amazonas - Brazil). Saplings of 6 native Amazonian species belonging to three distinct successional groups (Table 1) were cultivated in nursery and when they reached 9 months of age were transplanted to plastic pots containing 12 liters of substrate (regional latosoil collected in native forest with organic matter – see supplementary material for chemical characteristics). At 12-14 months of age, one part was transferred to 2 different environments of irradiance and one part was kept in the nursery (4 individuals per specie in each treatment). Incident photosynthetic radiation was monitored with a line quantum sensor (model LI-191, LI-COR Inc., Lincoln, Nebraska, USA) for 7 sunny days (see appendix for details).

Table 1: List of studied species with scientific name, family and successional group as described in the literature.

Specie	Family	Sucessional group	Reference
Hymenea courbaril	Fabaceae	Late-sucessional	Souza et al. (2009)
Carapa guianensis	Meliaceae	Late-sucessional	Vinson et al. (2005)
Hevea brasiliensis	Euphorbiaceae	Mid-sucessional	Amaral et al. (2009)
Tabebuia serratifolia	Bignoniaceae	Mid-sucessional	Gualberto et al.(2014)
Bellucia grossularioides	Melastomataceae	Pionner	Bentos et al. (2017)
Ochroma pyramidale	Malvaceae	Pionner	Slot and Winter (2018)

The light treatments (Table 2) consisted of full sunlight (FS) (100 % of solar radiation, simulating a forest clearing), artificial moderate shade (MS) provide by shade cloths reducing direct incident solar radiation (simulating an understory light environment with partial canopy openness) and natural deep shade (DS) with natural shade provided by adult tree canopies (simulating an understory light environment). The plants were kept in these environments during the 180 days of the experiment.

Table 2: Daily PAR average, maximum PAR observed, average percentage of full sunlight under three light environments.

Specie	Full Sunlight (FS)	Moderate Shade (MS)	Deep Shade (DS)
PAR average	1027.51±10.49	362.50 ± 9.37	47.95 ± 7.98
Maximum PAR	1866.17 (13:00)	795.61 (12:00)	74.50 (14:00h)
% PAR	100	35.23	4.66

2.2 Light and CO_2 curve responses

Leaf gas exchange and chlorophyll *a* fluorescence were measured simultaneously using an open-flow infrared analyzer system equipped with a leaf chamber fluorometer (LI-6400XT, Li-

Cor, Lincoln, NE, USA). The measurements were taken between 7:00 a.m. and 1:00 p.m. to determinate net photosynthetic rate (A), dark respiration (R_d), transpiration rate (E), intracellular carbon (C_i) and stomatal conductance (g_s). The fixed parameters were the flux (400 μmol s^{-1}), temperature (30 °C) and relative humidity between around 60%.

The photosynthetic response to irradiance (A/PPDF curves) was measured with a CO_2 concentration of 400 μmol mol^{-1} and 14 photosynthetic photon flux density (PPFD) levels (0, 10, 25, 50, 75, 100, 150, 200, 300, 500, 750, 1000,1500 and 2000 μmol m^{-2} s^{-1} in decreasing order). The A/PPFD curves were fitted with a non-rectangular hyperbola (Marshall and Biscoe, 1980; Santos Junior et al., 2013) to obtain the maximum photosynthetic rate (A_{max}) and apparent quantum yield (α):

$$A = \frac{\phi_{(PPDF_o)} \cdot PPDF + A_{max} - \sqrt{\left(\phi_{(PPDF_o)} \cdot PPDF + A_{max}\right)^2 - 4\theta \cdot \phi_{(PPDF_o)} \cdot PPDF \cdot A_{max}}}{2\theta} - R_d$$

The light compensation point (LCP) and light saturation point (LSP) were obtained from the light response curves. The LSP was estimated as the PPDF in 90% of A_{max} and LCP was calculated by equation:

$$LCP = \frac{Rd \times \left(\theta \times R_d - A_{max}\right)}{\phi_{(PPPDF_o)} \times \left(Rd - A_{max}\right)}$$

In light-adapted leaves, the actual quantum yield of PSII (ϕPSII) was determined following the procedures of Genty et al. (1989) and the photorespiratory rate of Rubisco (R_P) was obtained according to Valentini et al. (1995).

The electron transport rate (ETR) was calculated as:

$$\mathrm{ETR} = \alpha\beta \times \mathrm{PPDF} \times \phi\mathrm{PSII}$$

where PPFD is the photosynthetically active photon flux density, α is the leaf absorptance and βis the PSII optical cross section. The product $\alpha\beta$ was herein determined from curves obtained under non-photorespiratory conditions in an atmosphere containing less than 1% O_2 (Yin et al., 2009).

Photosynthetic responses to CO_2 concentration (A/C_i curves) were determined for a PPFD of 1500 μmolm^{-2} s^{-1} (saturating, but not photoinhibitory light) and 15 CO_2 levels (400, 350, 250, 150, 100, 75, 50, 400, 600, 800, 1000, 1300, 1500, 1800 and 2000 μmol mol^{-1}). Nonlinear regression techniques based on the equations of Farquhar et al. (1980) were used to calculate V_{cmax} and J_{max} for the curve A/C_i obtained. The constants used were the same as described in Sharkey et al.(2007).

The mesophyll conductance (g_m) was estimated by Harley et al.(1992):

$$g_m = \frac{A}{C_i - \frac{T^*[(ETR/4+2(A+R_d)]}{ETR/4-(A+R_d)}}$$

From g_m, partial pressure of CO_2 at the sites of carboxylation (C_c) was calculated following the equation:

$$C_c = C_i - \frac{A}{g_m}$$

A/C_i curves were also performed on dead leaves (after boiling, with ETR close to zero) to correct any CO_2 leaks inside the chamber of the gas analyzer, according to Flexas et al.(2007).

2.3 Photosynthetic limitations

The overall photosynthetic limitations were partitioned into their leaf functional components - stomatal (SL), mesophyll (ML) and biochemical (BL) limitations - using the calculated parameters g_s, g_m, chloroplastic CO_2 concentration (C_c),T*, K_m and V_{cmax}, following procedures described in Grassi and Magnani (2005):

$$SL = \frac{\frac{g_{tot}}{g_{s\_CO2}} \times \frac{\delta A}{\delta C_c}}{g_{tot} + \frac{\delta A}{\delta C_c}}$$

$$ML = \frac{\frac{g_{tot}}{g_m} \times \frac{\delta A}{\delta C_c}}{g_{tot} + \frac{\delta A}{\delta C_c}}$$

$$BL = \frac{g_{tot}}{g_{tot} + \frac{\delta A}{\delta C_c}}$$

where $g_{s\_CO_2}$ is the stomatal conductance to CO_2 ($g_{s\_CO2}=g_s/1.6$), g_m is the mesophyll diffusion conductance according to Harley et al. (1992) and g_{tot} is the total conductance to CO_2 from ambient air to chloroplasts was determined as:

$$g_{tot} = \frac{1}{\frac{1}{gs_{\_CO2}} + \frac{1}{g_m}}$$

and $\delta A/\delta C_c$ was calculated as:

$$\frac{\delta A}{\delta C_c} = \frac{V_{cmax} \times (T^* + K_m)}{(C_c + K_m)^2}$$

2.4 Partition of leaf nitrogen

The fractions of N in Rubisco (carboxylation - P_r), electron transport chain proteins (bioenergetics - P_b) and pigments involved in the capture of light (P_l) were estimated according describe by Niinemets and Tenhunen (1997):

$$P_r = \frac{V_{cmax}}{6.25 \times 20.5 \times (1/SLA) \times N_m}$$

where 6.25 g Rubisco (g N in Rubisco) $^{-1}$ is the conversion factor of N in protein; 20.5 μmol CO_2 (g Rubisco) $^{-1}$ s^{-1} the specific activity of Rubisco; SLA the specific leaf area (m^{-2} kg^{-1}) and N_m the total N content per leaf mass basis (g kg^{-1}).

$$P_b = \frac{J_{max}}{8.06 \times 156 \times (1/SLA) \times X}$$

where 8.06 μmol cytochrome f (g N in electron transport components)$^{-1}$ a conversion factor; and 156 mol electrons (mol cytochrome f)$^{-1}$ s^{-1} the electron transport activity factor per unit of cytochrome f.

$$P_l = \frac{C_m}{N_m \times C_B}$$

where C_m concentration of total chlorophylls, and C_B, is the weighted average of the amount of chlorophyll by the amount of nitrogen that is present in the photosystems (PSII and PSI) and in the PSII antennas (LHCII). The concentration of each enzyme complex per unit area and the ratio of each enzyme complex to the total concentration were calculated according to Hikosaka and Terashima (1995).

From the values of P_r and P_b and P_l was possible to determine the fraction of N in structural components (P_s) according to the equation:

$$P_s = 100 - P_r - P_b - P_l$$

2.5 Photosynthetic induction curve

The photosynthetic light induction curves were obtained using the using Li-6400 in the same settings as described above, based on the protocol described by Bai et al. (2008) and Martins et al. (2013), with some modifications.

To the totally dark-adapted leaves (at least 6hours), a weak modulated measuring beam (0.03 μmol m^{-2} s^{-1}) was applied to obtain the minimal fluorescence (F_0). The maximum fluorescence emissions (F_m) were measured after applying a saturating white light pulse of 8,000 μmol m^{-2} $s^{-1}$for 0.8 s. Then, leaf samples were then subjected to a PAR of 20 μmol m^{-2} s^{-1} for 5

min (initial state). Thereafter, the PAR was suddenly increased to 1,500 µmol m^{-2} s^{-1}, after which the rates of gas exchange (A, g_s, and C_i) were logged at 15s intervals and stored as 1 min averages over 35 min (induction time). During the induction time, the fluorescence signals F_s (steady-state fluorescence under actinic illumination of 1,500 µmol m^{-2} s^{-1}), F_m' (maximum fluorescence during a light-saturating pulse of 8,000 µmol m^{-2} s^{-1}) and F_0' (light-adapted minimal fluorescence, obtained using a weak far-red illumination) were measured and logged at 2 min intervals. After the induction time, the leaf tissues were again subjected to a PAR of 20 µmol m^{-2} s^{-1}, but for 15min and then exposed to a PAR of 1,500 µmol m^{-2} s^{-1} for 1 min in order to estimate the loss of photosynthetic induction. The light source was subsequently turned off for 15 min, after which both F_0 and F_m were measured as described above.

We calculated: initial and maximum A and g_s values; internal CO_2 concentration (C_i); the time to reach 90% A_{max} ($t_{90\%}$ A); dark respiration rates (R_d); the induction state (IS); initial and maximum electron transport rates (ETR) and the maximum quantum yield of photosystem II calculated as:

$$F_v/F_m = \frac{F_m - F_0}{F_m}$$

Additionally, the energy absorbed by photosystem II, as reflected by three yield components for dissipative processes (ϕPSII, the yield of photochemistry; ϕNPQ, the yield for dissipation by down-regulation; and ϕNO, the yield of other non-photochemical losses), was calculated as described by Kramer et al. (2004).

The limitations to photosynthesis throughout the induction curves were calculated using the model proposed by Woodrow and Moot (1989). In this model, stomatal limitations (SL) to photosynthesis are artificially removed via the normalization of photosynthetic rates for a constant C_i. The A values without SL (A*) were estimated as:

$$A^* = \frac{(A+R_d)\, x\, (C_{if} - T^*)}{C_i - T^*} - R_d$$

where C_{if} describes the C_i values at the end of the induction period and T* is the CO_2 compensation point in the absence of mitochondrial respiration. Stomatal and biochemical limitations (BL) were calculated as:

$$SL = \frac{A^* - A}{A_{max} + R_d} \qquad BL = \frac{A_{max} - A^*}{A_{max} + R_d}$$

where A_{max} is the maximum A at the end of the induction period. The total limitations to photosynthesis were calculated as the sum of SL and BL.

2.6 Specific leaf area and leaf mass area

Specific leaf area (SLA) was determined by the ratio between the leaf area and the leaf dry mass at 65 °C from leaf discs of a known area. Leaf mass area (LMA) is the opposite, the ratio between the leaf dry mass and the leaf area (Evans and Poorter, 2001).

2.7 Nutrients and photosynthetic nutrient use-efficiency

The leaf samples were dried in an oven at 65 °C to mass constant. The total N was determined by the Kjeldahl method. Macronutrients (Ca, Mg, P and K) and micronutrients (Fe, Zn, Cu and Mn) were extracted by digestion with 3:1 nitric-perchloric solution, concentrations of these nutrients was determined by atomic absorption spectrometry (Perkin-Elmer 1100B, Uberlingen, Germany) (Miyazawa et al., 1999) and P was determined by spectrophotometry at 725 nm (Vitti and Ferreira, 1997).

2.8 Experimental design and statistical analysis

The experimental design was factorial completely randomized (6X3) with six species in three treatments. After the assumptions of normality and homoscedasticity were complied, to analyze the differences between species and treatments, the appropriate analyses of variance and the averages were performed using the Tukey post hoc test. When appropriate, relationships between variables were tested by regression equations, using, as criteria for adjustment: 1) significance of the adjusted regression; 2) significance of the adjusted regression coefficients and 3) higher coefficient of determination.

The interrelationships among 34 functional traits involved in leaf gas exchange variables were assessed using the principal components analysis (PCA) ordination method. Product-moment correlations were used to assess the influences of light environment (full sunlight (FS), moderate shade (MS) and deep shade (DS)) on the ordination axes and each original variable and the correlations between each leaf trait and maximum photosynthesis.

All analyses were performed with Statistica 8.0 (Statsoft Inc. 2007), SPSS 23 (IBM Corp. 2015) and SigmaPlot 11 (Systat software, 2008).

3 RESULTS

3.1 Light and CO_2 curve responses

The pioneers *B. grossularioides* and *O. pyramidale* exhibited in FS higher values of A_{max} than all other species. Compared with MS, values were 1.7 and 1.3 times higher and, with DS, the first was 1.9 higher. After 97 days of the experiment, all of the *O. pyramidale* samples in DS were already dead. *H. courbaril* displayed higher A_{max} in MS, while *T. serratifolia* showed no difference between FS an MS. One exception was *C. guianensis* and *H. brasiliensis*, since they revealed no difference among treatments. All other species were lower in A_{max} in DS (Table 3). The lower A_{max} in DS was observed in *T. serratifolia*, however, all other species did not show any difference.

For other parameters, such as E, g_s, LSP, ETR and R_P, the pioneer species *B. grossularioides* and *O. pyramidale* displayed higher values in FS. *C. guianensis* exhibited LSP similar to pioneers in this environment. In MS, higher values of these parameters were observed for *H. courbaril*, followed by *O. pyramidale* and *T. serratifolia*. For DS, lower E and g_s were displayed for *H. courbaril*, while *B. grossularioides* displayed lower LSP, ETR and R_p. If compared to FS, with exception of *H. brasiliensis*, all plants in DS displayed lower values for LSP, ETR and R_P. Considering g_m, most of species displayed higher values in FS, only *C. guianensis* and H. *brasiliensis* were higher in DS (+24.7%) and (+27.9%) (Table 3).

As expected, in DS, LCP were lower for all species; remarkably so in *H. courbaril* and *C. guianensis* (Figure 2). This reduction in LCP was correlated with R_d (R^2 = 0.68), as is demonstrated in Figure 1. Lower R_d in FS were displayed for *H. brasiliensis* (-40.2% than MS), while in DS *T. serratifolia* (-69.6%) and *B. grossularioides* (-52.9%) were also lower than MS.

The effect of light on plants in low O_2 conditions can be observed in Figure 1, where the increase of A under saturating light is noticed, however, there was a reduction of LSP, which denotes the importance of photochemical processes, such as photorespiration for energy dissipation, and maintains high photosynthetic rates for longer in conditions of high irradiance.

Table 3: Maximum photosynthesis ($A_{máx}$), transpiration rate (E), stomatal conductance (g_s), mesophyll conductance (g_m), light saturation point (LSP), electrons transport rate (ETR) and photorespiration rate (R_P) in saplings of six tree species submitted to three different light environments.

ENVIRONMENT	SPECIES	A_{max} (µmol CO_2 $m^{-2}s^{-1}$)	E (µmol H_2O $m^{-2}s^{-1}$)	g_s (µmol CO_2 $m^{-2}s^{-1}$)	g_m (µmol CO_2 $m^{-2}s^{-1}$)	LSP (µmol PPDF $m^{-2}s^{-1}$)	ETR (µmol e^- $m^{-2}s^{-1}$)	R_P (µmol CO_2 $m^{-2}s^{-1}$)
FULL SUNLIGHT	*H. courbaril*	9.2 ± 1.9^{BCab}	2.2 ±0.5^{BCDa}	58.2 ± 5.0 ^{Cb}	76.35 ± 2.01^{ABa}	437.1 ± 86.8 ^{BCa}	72.8 ±7.9 ^{Bca}	3.3 ± 0.2 ^{Ba}
	C. guianensis	5.7 ± 1.0^{Ca}	1.5 ± 0.2 ^{Da}	44.4 ± 5.3^{Ca}	37.61 ± 8.54^{Cab}	604.1 ± 111.9 ^{ABa}	63.6 ± 8.8 ^{Ca}	3.3 ± 0.5 ^{Ba}
	H. brasiliensis	7.8 ±1.1^{BCa}	1.9 ± 0.5^{CDa}	64.0 ±29.6 ^{BCa}	56.96 ± 13.34^{BCab}	388.4 ± 83.7 ^{Ca}	70.6 ± 7.1 ^{BCa}	3.6 ± 0.4 ^{Ba}
	T. serratifolia	10.3 ± 1.6^{ABCa}	2.7 ± 0.3^{BCa}	69.1 ± 16.7 ^{BCb}	56.89 ± 4.81^{BCb}	474.6 ±54.7 ^{BCa}	86.1 ± 6.3 ^{Ba}	4.0 ± 0.1 ^{ABa}
	B. grossularioides	15.5 ± 4.4^{Aa}	4.1 ±0.4^{Aa}	182.6 ± 17.2 ^{Aa}	73.87 11.13^{ABa}	588.6 ±96.4 ^{ABa}	123.2 ±14.9 ^{Aa}	4.8 ± 0.9 ^{Aa}
	O. pyramidale	12.4 ± 2.5^{ABa}	3.1 ± 0.7^{ABa}	141.9 ± 79.4^{ABa}	89.74 ± 17.76^{Aa}	700.2 ±60.0 ^{Aa}	107.0 ± 4.7 ^{Aa}	5.9 ± 0.6 ^{Aa}
MODERATE SHADE	*H. courbaril*	11.9 ± 2.5^{Aa}	3.4 ± 0.8 ^{Aa}	126.7 ± 17.2 ^{Aa}	64.69 ± 6.52^{ABa}	492.0 ± 51.8 ^{Aa}	88.4 ± 11.5 ^{Aa}	3.8 ± 0.4 ^{Aa}
	C. guianensis	7.3 ± 1.3 ^{Ba}	1.7 ± 0.4 ^{Ba}	55.7 ± 16.9 ^{Ca}	49.54 ± 13.83^{BCa}	393.3 ± 80.3 ^{ABb}	50.3 ± 9.1 ^{Bab}	2.0 ± 0.4 ^{Cb}
	H. brasiliensis	7.0 ± 1.5 ^{Ba}	1.7 ± 0.3 ^{Ba}	54.0 ± 10.0 ^{Ca}	49.92 ± 2.60^{BCb}	318.7 ± 16.5 ^{Ba}	52.0 ± 12.9 ^{Bb}	2.2 ±0.6 ^{BCb}
	T. serratifolia	10.5 ± 2.2^{ABa}	3.2 ± 1.3 ^{ABa}	111.9 ± 8.0^{ABa}	74.02 ± 1.50^{ABa}	406.5 ± 48.5 ^{Aba}	83.7 ± 7.8 ^{Aa}	3.3 ± 0.5 ^{ABb}
	B. grossularioides	9.1 ± 2.0^{ABb}	2.2 ±0.4 ^{ABb}	81.3 ± 14.9 ^{BCb}	31.64 ± 2.54^{Cb}	429.0 ± 54.9 ^{ABb}	69.3 ± 10.3 ^{ABb}	2.6 ± 0.6 ^{BCb}
	O. pyramidale	9.1 ± 0.9 ^{ABb}	2.8 ± 0.4 ^{ABa}	115.2 ± 35.9 ^{ABa}	81.95 ± 3.89^{Aa}	490.9 ± 5.7 ^{Ab}	68.9 ± 3.3 ^{ABb}	3.1 ± 0.4 ^{ABCb}
DEEP SHADE	*H. courbaril*	5.7 ± 1.7 ^{ABb}	0.7 ± 0.2 ^{Bb}	13.2 ± 5.3 ^{Bc}	36.83 ± 0.23^{Bcb}	256.5 ± 21.8 ^{ABb}	39.6 ± 9.8 ^{BCb}	1.7 ± 0.2 ^{ABb}
	C. guianensis	5.8 ± 0.3^{ABa}	1.4 ± 0.3 ^{ABa}	52.7 ± 5.9 ^{Aa}	29.08 ± 10.02^{Cb}	331.9 ± 46.1 ^{ABb}	42.6 ± 8.3 ^{BCb}	1.7 ± 0.5 ^{ABb}
	H. brasiliensis	7.3 ± 1.2 ^{ABa}	1.4 ± 0.2 ^{Aa}	53.0 ± 18.5 ^{Aa}	79.01 ± 19.94^{Aa}	389.8 ± 79.3 ^{Aa}	62.7 ± 5.8 ^{Aab}	2.5 ± 0.7 ^{Aab}
	T. serratifolia	5.1 ± 0.7 ^{Bb}	1.5 ± 0.4 ^{Ab}	69.9 ± 18.7^{Ab}	56.43 ± 13.49^{ABb}	195.7 ± 82.0 ^{Bb}	36.5 ± 1.8 ^{Cb}	1.4± 0.2 ^{Bc}
	B. grossularioides	8.2 ± 1.5^{Ab}	1.9 ± 0.5 ^{Ab}	46.0 ± 16.1 ^{Ac}	42.12 ± 6.58^{BCb}	404.6 ± 135.2 ^{Ab}	55.8 ± 9.1 ^{ABb}	2.2 ± 0.3 ^{ABb}
	O. pyramidale							

Mean ± standard deviation (n=4) follow in lines for same capital letter to different species in same environment and lower case to same species in different environment are equal by Tukey test ($p<0.05$)

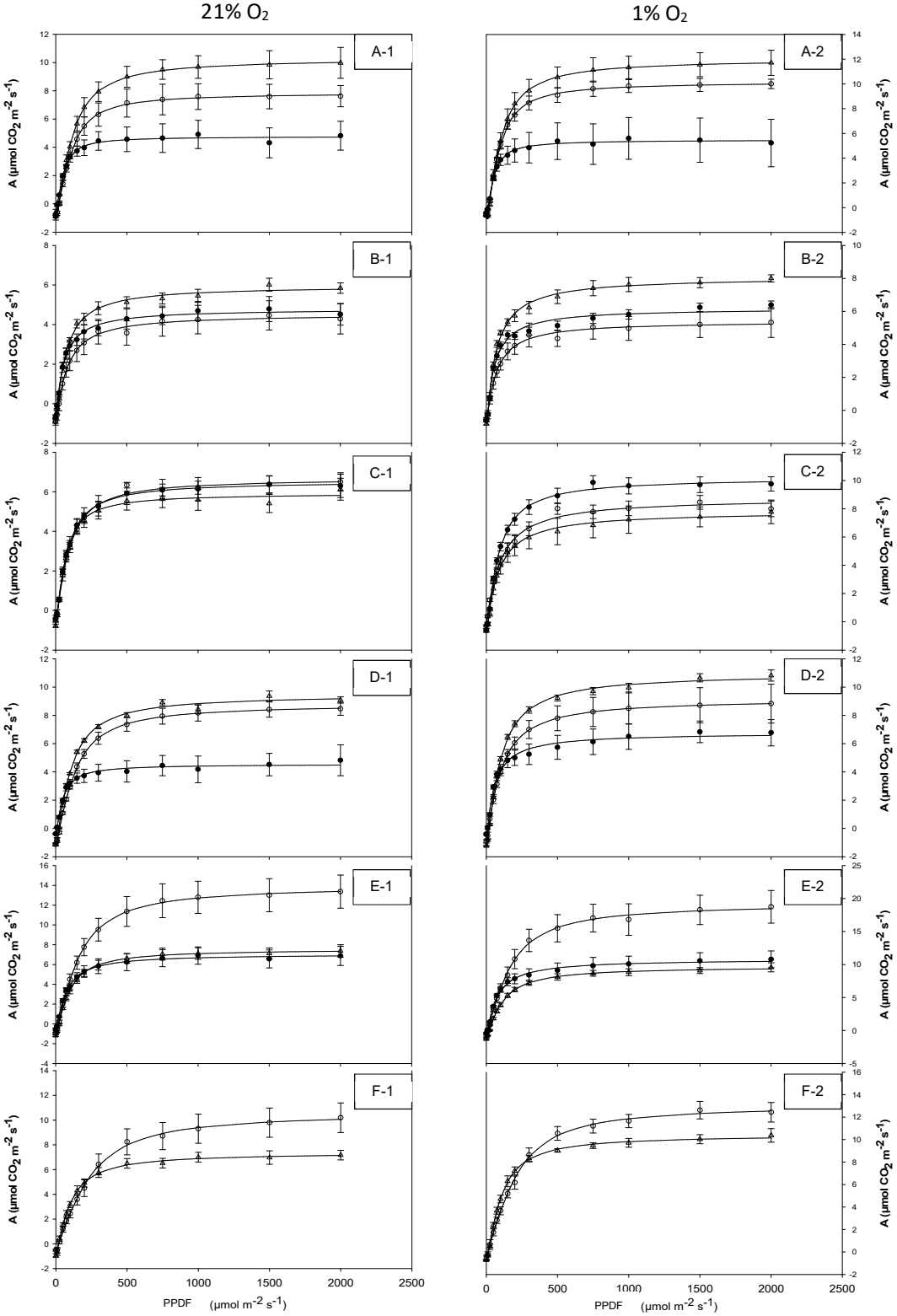

Figure 1: Light curve response for six tree species: A) *Hymenea courbaril*, B) *Carapa guianensis*, C) *Hevea brasiliensis*, D) *Tabebuia serratifolia*, E) *Bellucia grossularioides* and F) *Ochroma pyramidale* submitted to three light environments: FS - full sunlight (○); MS – moderate shade (Δ) and DS - deep shade (●) and two O_2 levels: 21% and 1% (columns 1 and 2, respectively).

In general, between the environments, J_{max} and V_{cmax} in area basis have decreased with the decrease of the irradiance, while in mass basis it was the inverse. However, among species, in contrast to V_{cmax}, J_{max} similar behavior did not occur between the area base and the mass basis in MS and DS. The similarities were only the lowest values for *C. guianensis* and *B. grossularioides* in MS and *C. guianensis* and *H. courbaril* in DS. While in the base area *H. courbaril* exhibited V_{cmax} almost 2 times greater than *B. grossularioides*, in mass basis they did not differ between them and both were similar to *H. brasiliensis* and *T. serratifolia*. For the of ratio J_{max}/V_{cmax}, except for *H. brasiliensis* (-15.6%) and *C. guianensis*, which showed no difference, the DS plants exhibited values greater or at least equal to the FS plants (Table 4).

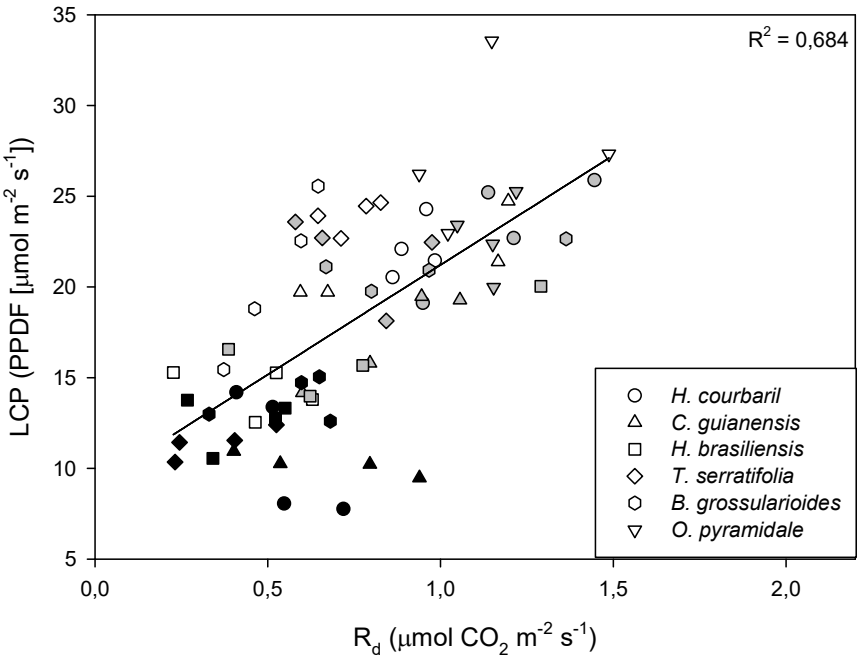

Figure 2: Dispersion graph with relationship between light compensation point (LCP) in relation to dark respiration (Rd) for saplings of six tree species subjected to three different light environments: full sunlight (open symbols), moderate shade (gray symbols) and deep shade (black symbols).

The highest V_{cmax} and J_{max} values, both in area and mass basis, were observed for the pioneer species in FS (Table 4). However, for the same variables, *O. pyramidale* shows higher values than *B. grossularioides* (+11.3% and +17.3% in area and +59% and +61.8% in mass basis). The high photosynthetic rates of these species in FS can be attributed, in part, to the high rate of carboxylation under high irradiance. As the irradiance decreased, V_{cmax} for the pioneer species also decreased, so that the highest value observed in MS and DS was for *H. courbaril*. Surprisingly, *H. brasiliensis* did not show variations in the different environments and, for *C. guianensis,* Vc_{max} was similar between FS and DS, revealing a higher value in MS (+32.1%) (Table 4).

Table 4: Maximum carboxylation (V_{cmax}) and electrons transport (J_{max}) rates per area (a) and mass (m) unit and ratio between maximum carboxylation and maximum electron transport in saplings of six tree species submitted to three different light environments.

ENVIRONMENT	SPECIES	V_{cmax}_a ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	J_{max}_a (μmol electrons $m^{-2}\ s^{-1}$)	V_{cmax}_ m ($\mu mol\ CO_2\ g^{-1}\ s^{-1}$)	J_{max}_ m (μmol electrons $g^{-1}\ s^{-1}$)	J_{max}/V_{cmax}
FULL SUNLIGHT	*H. courbaril*	54.2 ± 3.2^{Cb}	69.7 ± 5.4^{Cb}	0.7 ± 0.1^{BCb}	0.9 ± 0.1^{Bcb}	1.3 ± 0.03^{Bb}
	C. guianensis	28.0 ± 0.6^{Eb}	36.8 ± 2.3^{Db}	0.3 ± 0.04^{Cb}	0.4 ± 0.1^{Cb}	1.3 ± 0.1^{Ba}
	H. brasiliensis	42.5 ± 5.2^{Da}	65.7 ± 12.6^{Ca}	0.7 ± 0.3^{Bb}	1.2 ± 0.5^{Bb}	1.5 ± 0.2^{Aa}
	T. serratifolia	47.2 ± 4.0^{CDb}	61.8 ± 4.8^{Cb}	0.8 ± 0.3^{Bb}	1.1 ± 0.4^{Bb}	1.3 ± 0.1^{Ba}
	B. grossularioides	91.4 ± 3.1^{Ba}	122.5 ± 9.0^{Ba}	1.0 ± 0.1^{Ba}	1.3 ± 0.1^{Ba}	1.3 ± 0.1^{Bb}
	O. pyramidale	103.1 ± 0.6^{Aa}	147.8 ± 5.6^{Aa}	2.4 ± 0.1^{Aa}	3.5 ± 0.2^{Aa}	1.4 ± 0.04^{ABa}
MODERATE SHADE	*H. courbaril*	81.6 ± 5.4^{Aa}	98.5 ± 3.2^{Aa}	1.4 ± 0.5^{Aa}	1.6 ± 0.5^{ABa}	1.2 ± 0.1^{BCb}
	C. guianensis	40.3 ± 1.2^{DEa}	47.9 ± 4.0^{Ea}	0.6 ± 0.1^{BCa}	0.7 ± 0.05^{Bab}	1.2 ± 0.1^{Ca}
	H. brasiliensis	47.4 ± 3.3^{CDa}	61.6 ± 5.5^{CDa}	1.0 ± 0.4^{ABCb}	1.3 ± 0.6^{ABb}	1.3 ± 0.1^{BCb}
	T. serratifolia	62.3 ± 3.4^{Ba}	80.6 ± 2.3^{Ba}	1.4 ± 0.2^{Aa}	1.8 ± 0.3^{Aa}	1.3 ± 0.05^{BCa}
	B. grossularioides	33.0 ± 2.0^{Eb}	53.8 ± 2.8^{DEb}	0.5 ± 0.1^{Cc}	0.8 ± 0.1^{Bb}	1.6 ± 0.1^{Aa}
	O. pyramidale	48.8 ± 4.8^{Cb}	67.8 ± 4.8^{Cb}	1.3 ± 0.4^{ABa}	1.8 ± 0.7^{Ab}	1.4 ± 0.1^{Ba}
DEEP SHADE	*H. courbaril*	19.4 ± 5.0^{Cc}	28.8 ± 7.0^{Dc}	0.7 ± 0.03^{Bb}	1.0 ± 0.1^{BCb}	1.5 ± 0.1^{Aba}
	C. guianensis	26.8 ± 1.5^{Bb}	34.6 ± 4.5^{CDb}	0.7 ± 0.2^{Ba}	0.9 ± 0.3^{Ca}	1.3 ± 0.1^{BCa}
	H. brasiliensis	44.7 ± 2.8^{Aa}	58.3 ± 3.9^{Aa}	1.9 ± 0.2^{Aa}	2.4 ± 0.3^{Aa}	1.3 ± 0.01^{BCb}
	T. serratifolia	32.8 ± 2.0^{Bc}	41.5 ± 3.7^{BCc}	1.7 ± 0.1^{Aa}	2.2 ± 0.2^{Aa}	1.3 ± 0.04^{Ca}
	B. grossularioides	30.8 ± 1.8^{Bb}	48.7 ± 3.0^{ABb}	0.9 ± 0.1^{Bb}	1.4 ± 0.2^{Ba}	1.6 ± 0.2^{Aa}
	O. pyramidale					

Mean ± standard deviation (n=4) follow in lines for same capital letter to different species in same environment and lower case to same species in different environment are equal by Tukey test ($p<0.05$).

3.2 Photosynthetic limitations

The overall photosynthetic limitations were essentially more balanced in FS than other environments (30.0%, 38.1% and 31,9% in FS against 25.9%, 39.3% and 34.9% in MS and 33.6%, 27.5% and 39% in DS for stomatal (SL), mesophyll (ML) and biochemical (BL) limitations, respectively (Figure 3A-C). In general, diffusive limitations (SL+ML) were the main restrictions to photosynthesis. Nevertheless, BL were more pronounced in *O. pyramidale* in FS and MS (41.1% and 46.8%, respectively) and *C. guianensis*, *H. brasiliensis* and *T. serratifolia* in DS (48.5%, 42.8% and 47.3%).

For SL, *H. brasiliensis* and *O. pyramidale* did not differ between FS and MS. *C. guianensis* and *T. serratifolia* were 40.5% and 14.9% higher in FS than in DS and, unlike these, *H. courbaril* and *B. grossularioides* were 1.4 and 1.8 times higher in DS than in FS, respectively (Figure 3A-C). Concerning ML, greatest values were observed for *B. grossularioides* in FS and MS that were 32.9% higher than those in DS. In addition, lower ML was observed for all species of plants in DS (Figure 3A-C).

3.3 Partition of leaf nitrogen

The partition of leaf nitrogen reflected the different strategies of the species in relation to the environments. In FS, the pioneers *B. grossularioides* and *O. pyramidale* invested most of the leaf nitrogen in rubisco (P_r = 64% and 62%, respectively) and bioenergetics (P_b = 13% and 10%, respectively), which was certainly decisive for the high Vc_{max} and J_{max} of these species (Figure 1D). The non-pioneers, on the other hand, exhibited P_s higher than 50% in this environment; more than 3 times higher than the pioneers.

Contrasting with FS, in MS, *H. courbaril* (a late successional) and *O. pyramidale* (a pioneer) showed similar behavior regarding leaf nitrogen partition (Figure 3E).

The investment in pigments involved in light capture was clearly higher in DS for all species (Figure 3F). The highest balance between P_r, P_l and P_s in this environment is highlighted, considering that in FS and MS more than 80% of the nitrogen was partitioned between P_r and P_s. Only *C. guianensis* and *H. brasiliensis* exhibited lower P_s in DS than in FS.

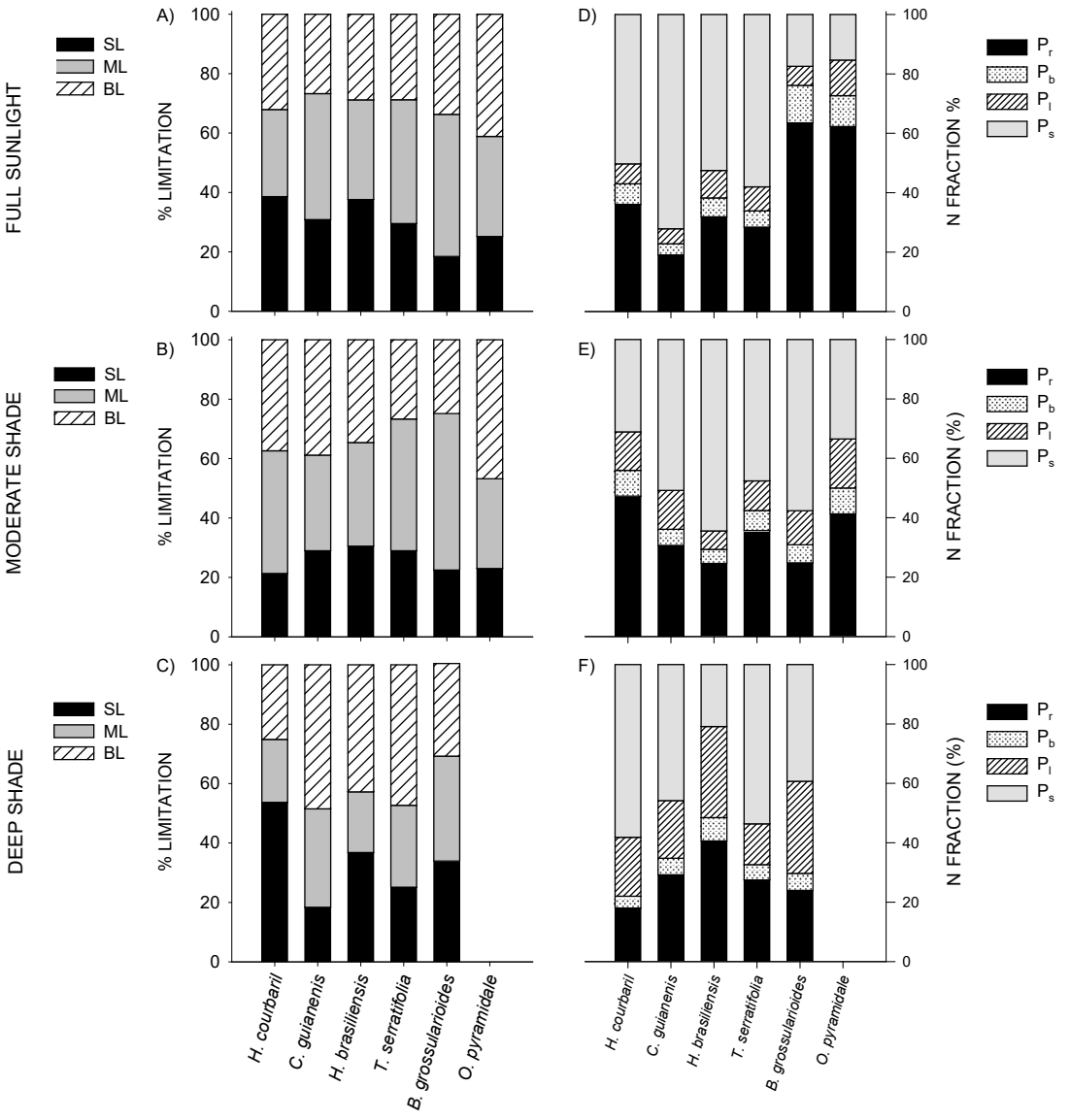

Figure 3: Stomatal (SL) mesophilic (ML) and biochemical (BL) photosynthetic limitations and leaf nitrogen partition in Rubisco (P_r), bioenergetics (P_b), light capture (P_l) and structural components (P_s) in saplings of six tree species submitted to three different light environments: full sunlight (A and D), moderate shade (B and E) and deep shade (C and F).

3.4 Photosynthetic induction curve

The photosynthetic induction curves followed a more evident pattern in MS and DS leaves than in FS, which was well-marked by the lower initial activity of late-successional species in comparison to the pioneers (Figure 4 A1-3).

The highest A_{max} values were for the pioneers *B. grossularioides* and *O. pyramidale* in FS, which were probably benefited by larger A and g_s values at the beginning of the curve. These species also showed higher ETR at the beginning and at the end of the curve and reached 90% A_{max} and g_{smax} before the others (Table 5).

In general, plants in DS maintained higher IS% than plants in FS (+28.93% for *H. courbaril*, +26.8% for *C. guianensis*, +34.5% for *H. brasiliensis*, +77.5% for *T. serratifolia* and +31.7% for *B. grossularioides*). *O. pyramidale* displayed lower IS values in FS than all other plants and *B. grossularioides* was the worse in DS (Table 5 and Figure 4 E1-3).

The SL had lower influence on the first minutes of the curve in FS and MS than in DS, especially for non-pioneer species, so that the greatest restriction to photosynthesis occurred from BL (Figure 5).

Irrespective of treatments, rapid activation of the fluorescence parameters was observed after exposing dark acclimated leaf tissues to illumination, as demonstrated by the decrease in ϕNO and increase in ϕPSII during the first 15 min of photosynthetic induction curve for all species and environments. ϕNPQ remained unaltered throughout the induction curve in FS and MS, but showed a strong increase in DS (Table 6).

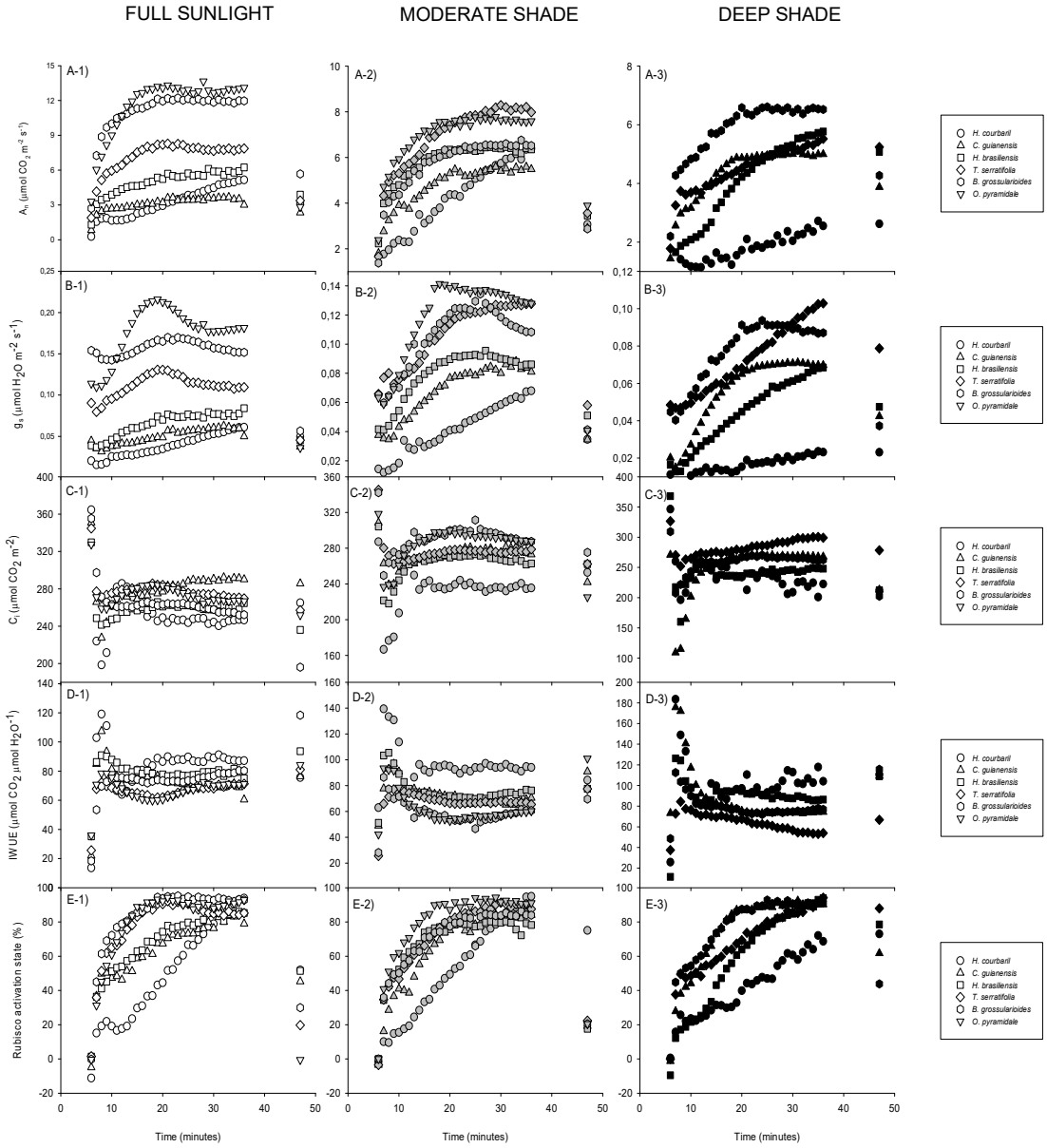

Figure 4: Course of photosynthetic parameters in induction curve: A) net photosynthesis (A_n), B) stomatal conductance (g_s), C) intracellular CO_2 concentration (C_i), D) intrinsic water efficiency use (IWUE) and E) Rubisco activation state (IS%) in saplings of six tree species submitted to three light environments: full sunlight (open symbols); moderate shade (gray symbols) and deep shade (black symbols). Dates are mean (n=4).

Table 5: Parameter of photosynthetic induction net photosynthesis initial (A_i) and maximum (A_{max}), time to reach 90% of maximum values ($T90\%X_{max}$), photosynthetic induction state after dark (IS%), stomatal conductance initial (g_{si}) and max (g_{smax}) and electrons transport rate initial (ETR_i) and final (ETR_f) in saplings of six tree species subjected to three light environments.

ENVIRONMENT	SPECIE	A_i	A_{max}	$T90\%A_{max}$	IS	g_{si}	g_{smax}	$T90\%g_s$	ETR_i	ETR_f
		($\mu mol\ CO_2\ m^{-2}s^{-1}$)		(minutes)	(%)	($\mu molH_2O\ m^{-2}s^{-1}$)		(minutes)	(μmol electrons $m^{-2}s^{-1}$)	
FULL SUNLIGHT	*H. courbaril*	0.78 ± 0.33^{Ca}	5.17 ± 1.30^{Ca}	29.91 ± 2.22^{Aa}	36.49 ± 10.63^{Ab}	20.68 ± 8.22^{Ba}	60.71 ± 19.26^{Ca}	32.10 ± 1.72^{Aa}	23.11 ± 1.86^{Cb}	40.30 ± 5.90^{ABa}
	C. guianensis	0.78 ± 0.30^{Cb}	4.38 ± 1.58^{Ca}	25.95 ± 7.26^{Aa}	56.10 ± 9.38^{Aa}	38.47 ± 18.90^{Ba}	61.56 ± 18.11^{Ca}	24.94 ± 3.81^{ABa}	11.66 ± 2.50^{Ca}	30.93 ± 10.36^{Ba}
	H. brasiliensis	1.32 ± 0.37^{BCa}	5.91 ± 1.20^{Ca}	22.13 ± 5.24^{ABab}	67.44 ± 16.35^{Ab}	38.52 ± 6.47^{Ba}	83.92 ± 13.70^{Ca}	21.86 ± 4.75^{BCa}	19.42 ± 4.09^{Ca}	43.93 ± 5.18^{ABa}
	T. serratifolia	2.03 ± 0.76^{ABa}	8.51 ± 2.19^{BCa}	13.95 ± 3.31^{Cb}	29.24 ± 11.66^{Ab}	91.00 ± 48.22^{ABa}	109.24 ± 22.30^{Bab}	15.31 ± 3.09^{Cb}	32.62 ± 0.71^{BCa}	45.01 ± 2.70^{ABa}
	B. grossularioides	2.71 ± 0.96^{ABa}	12.55 ± 1.88^{ABa}	14.98 ± 3.64^{Ca}	43.85 ± 9.45^{Aa}	154.13 ± 66.56^{Aa}	166.80 ± 14.01^{ABb}	17.86 ± 6.77^{BCa}	55.78 ± 8.77^{ABa}	61.90 ± 15.85^{Aa}
	O. pyramidale	3.09 ± 1.11^{Aa}	13.60 ± 4.09^{Aa}	13.69 ± 3.53^{Ca}	8.83 ± 3.46^{Bb}	111.10 ± 64.66^{ABa}	181.25 ± 40.74^{Aa}	16.07 ± 0.60^{BCa}	60.05 ± 9.75^{Aa}	61.68 ± 4.34^{Aa}
MODERATE SHADE	*H. courbaril*	0.93 ± 0.90^{Aa}	6.39 ± 1.73^{Aa}	30.61 ± 1.90^{Aa}	76.88 ± 7.24^{Aa}	14.29 ± 3.61^{Ca}	43.26 ± 12.37^{Ca}	31.23 ± 1.30^{Aa}	35.22 ± 0.67^{Aa}	41.47 ± 4.14^{Aa}
	C. guianensis	1.83 ± 0.29^{Aa}	5.67 ± 0.64^{Aa}	20.37 ± 4.68^{Ba}	19.47 ± 3.58^{Cb}	37.88 ± 7.2^{Ba}	74.89 ± 15.98^{Ba}	20.89 ± 5.85^{ABa}	8.14 ± 3.17^{Bab}	24.75 ± 2.67^{Ba}
	H. brasiliensis	2.23 ± 1.35^{Aa}	6.79 ± 0.57^{Aa}	18.15 ± 1.28^{Bb}	36.91 ± 9.38^{Bb}	41.64 ± 12.22^{ABa}	86.06 ± 17.56^{Ba}	18.48 ± 6.17^{Ba}	25.36 ± 4.13^{ABa}	33.92 ± 7.03^{ABa}
	T. serratifolia	1.65 ± 0.86^{Aa}	8.55 ± 1.91^{Aa}	21.06 ± 6.42^{Bab}	44.49 ± 4.06^{Bb}	65.84 ± 8.65^{Ab}	144.43 ± 29.95^{Aa}	22.13 ± 5.75^{ABa}	34.44 ± 6.09^{Aa}	39.09 ± 9.80^{Aa}
	B. grossularioides	1.37 ± 0.68^{Aa}	6.96 ± 0.90^{Ab}	18.11 ± 2.76^{Ba}	34.25 ± 17.99^{Ba}	65.26 ± 9.09^{Ab}	108.51 ± 15.47^{Aab}	20.01 ± 4.69^{Ba}	24.84 ± 1.30^{ABb}	28.60 ± 7.64^{ABb}
	O. pyramidale	2.49 ± 1.12^{Aa}	7.89 ± 1.50^{Ab}	14.40 ± 1.60^{Ba}	26.84 ± 7.17^{Ba}	60.20 ± 2.93^{Ab}	112.65 ± 2.39^{Ab}	16.71 ± 0.87^{Ba}	31.06 ± 9.52^{Ab}	40.10 ± 10.74^{ABb}
DEEP SHADE	*H. courbaril*	0.56 ± 0.45^{Ba}	2.68 ± 1.62^{Ba}	30.43 ± 4.37^{Aa}	79.79 ± 6.03^{Aa}	9.93 ± 5.90^{Ba}	27.28 ± 9.25^{Ca}	33.34 ± 2.56^{Aa}	7.69 ± 1.81^{Cc}	31.40 ± 1.22^{Bb}
	C. guianensis	1.41 ± 0.40^{ABab}	5.17 ± 0.54^{ABa}	16.60 ± 3.24^{Ca}	73.74 ± 9.13^{Aa}	19.73 ± 5.33^{Bb}	69.74 ± 15.33^{Ba}	18.97 ± 4.40^{Ba}	3.90 ± 1.82^{Cb}	27.44 ± 5.43^{Ba}
	H. brasiliensis	0.79 ± 0.58^{Ba}	5.85 ± 1.23^{Aa}	27.57 ± 4.69^{ABa}	78.52 ± 12.75^{Aa}	16.36 ± 6.16^{Bb}	68.14 ± 19.42^{Ba}	28.24 ± 5.80^{Aa}	17.65 ± 3.60^{Ba}	44.50 ± 5.76^{ABa}
	T. serratifolia	1.78 ± 0.56^{ABa}	5.53 ± 0.90^{Aa}	24.21 ± 5.19^{ABCa}	87.91 ± 7.85^{Aa}	48.48 ± 11.46^{Ab}	102.45 ± 14.81^{Ab}	29.28 ± 3.07^{Aa}	14.70 ± 3.12^{Bb}	34.05 ± 2.63^{Bb}
	B. grossularioides	2.20 ± 0.93^{Aa}	6.48 ± 1.33^{Ab}	17.91 ± 4.42^{BCa}	48.79 ± 5.49^{Ba}	44.75 ± 17.90^{Ab}	82.62 ± 23.33^{ABb}	19.39 ± 1.38^{Ba}	25.33 ± 3.24^{Ab}	38.04 ± 5.78^{ABb}
	O. pyramidale									

Mean ± standard deviation (n=4) follow in lines for same capital letter to different species in same environment and lower case to same species in different environment are equal by Tukey test ($p<0.05$)

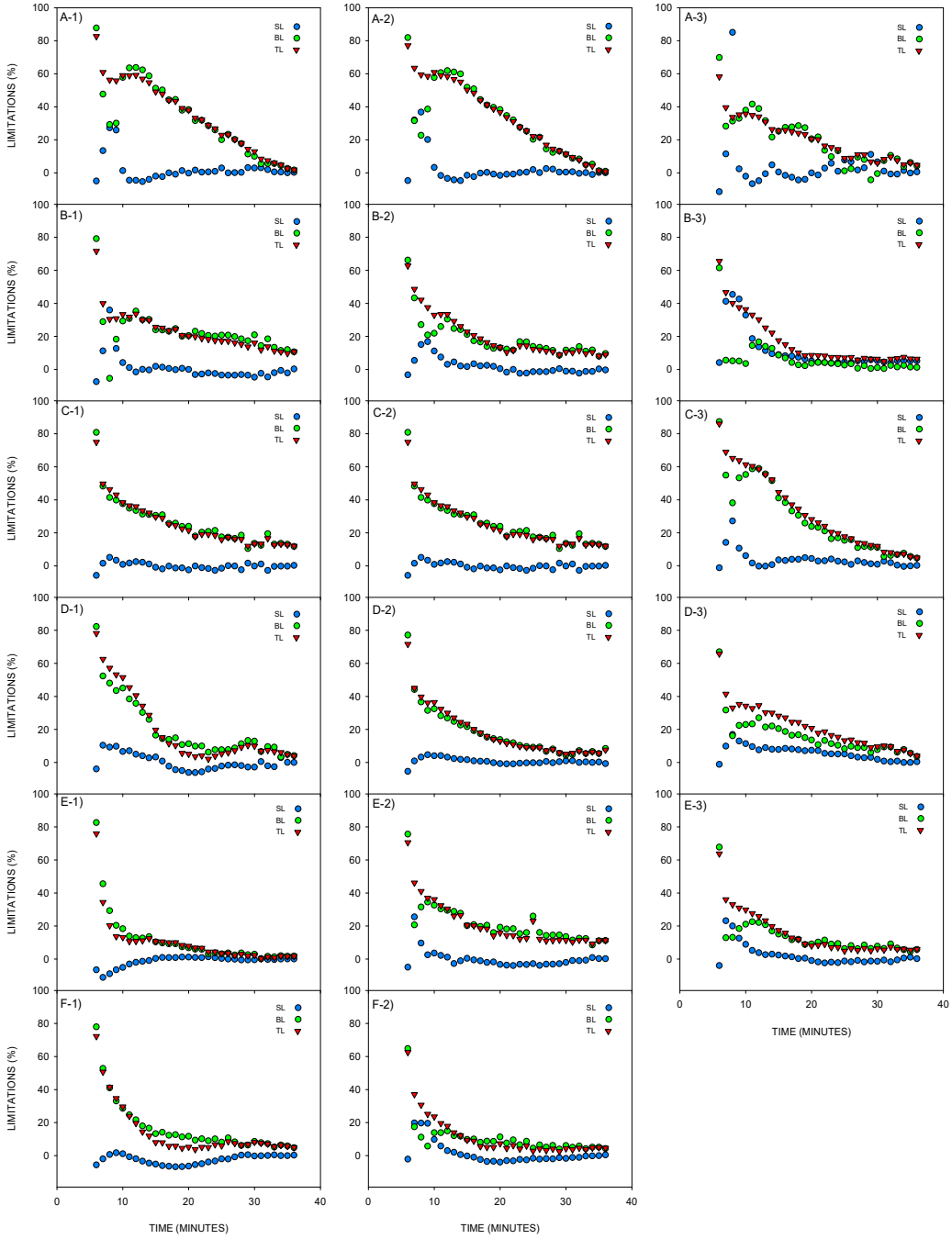

Figure 5: Stomatal (SL), biochemical (BL) and total (TL) limitations which drive photosynthesis during photosynthetic induction curve of six tree species saplings: *Hymenea courbaril*, *Carapa guianensis*, *Hevea brasiliensis*, *Tabebuia serratifolia*, *Bellucia grossularioides* and *Ochroma pyramidale* in three different light environments: full sunlight, moderate shade and deep shade (lines A, B, C, D, E and F; columns 1, 2 and 3, respectively).

Table 6: Fluorescence parameters in photosynthetic induction curves: Maximum PSII quantum yield at start (F_v/F_{mi}) and end of curve (F_v/F_{mf}), quantum yield of non-regulated energy dissipation in PSII (ϕNO), quantum yield for dissipation by down-regulation (ϕNPQ) and effective quantum yield of PSII (ϕPSII) at 1, 15 and 30 minutes of curve in saplings of six tree species subjected to three light environments.

ENVIRONMENT	SPECIE	F_v/F_{mi}	F_v/F_{mf}	ϕNO_{1min}	ϕNO_{15min}	ϕNO_{30min}	ϕNPQ_{1min}	ϕNPQ_{15min}	ϕNPQ_{30min}	$\phi PSII_{1min}$	$\phi PSII_{15min}$	$\phi PSII_{30min}$
FULL SUNLIGHT	*H. courbaril*	0.81±0.01^{Aa}	*0.67±0.03^{Aa}	0.28±0.01^{ABb}	*0.24±0.01^{Ab}	*0.23±0.01^{Ab}	0.69±0.01^{Aa}	0.68±0.01^{ABa}	0.67±0.01^{ABa}	0.04±0.00^{Cb}	*0.08±0.01^{Cab}	*0.10±0.01^{BCab}
	C. guianensis	0.72±0.02^{Bb}	*0.61±0.06^{Aa}	0.34±0.03^{ABa}	*0.22±0.01^{ABb}	*0.21±0.01^{ABb}	0.65±0.04^{Aa}	*0.72±0.02^{Aa}	*0.72±0.02^{Aa}	0.01±0.00^{Da}	*0.06±0.01^{Ca}	*0.07±0.02^{Ca}
	H. brasiliensis	0.78±0.03^{Aa}	*0.67±0.01^{Aa}	0.39±0.10^{Aa}	*0.22±0.02^{ABCb}	*0.21±0.02^{ABb}	0.58±0.10^{Aa}	*0.68±0.02^{ABa}	*0.68±0.02^{ABa}	0.03±0.01^{Ca}	*0.10±0.01^{BCa}	*0.10±0.01^{BCa}
	T. serratifolia	0.79±0.02^{Aa}	*0.65±0.0^{Ab}	0.27±0.07^{Bb}	*0.19±0.02^{Cc}	*0.18±0.02^{Bc}	0.68±0.07^{Aa}	0.70±0.01^{Aa}	0.70±0.01^{Aa}	0.05±0.01^{BCa}	*0.12±0.01^{BCa}	*0.12±0.02^{BCa}
	B. grossularioides	0.85±0.01^{Aa}	*0.68±0.01^{Aa}	0.24±0.01^{Bb}	*0.19±0.01^{Cb}	*0.18±0.01^{Bb}	0.67±0.01^{Aa}	0.66±0.03^{ABa}	0.67±0.04^{ABab}	0.08±0.01^{ABa}	*0.15±0.02^{ABa}	*0.15±0.03^{ABa}
	O. pyramidale	0.82±0.04^{Aa}	*0.64±0.04^{Aa}	0.25±0.03^{Bb}	*0.19±0.01^{BCb}	*0.19±0.02^{Bb}	0.66±0.03^{Aa}	0.62±0.06^{Ba}	0.62±0.07^{Ba}	0.09±0.04^{Aa}	*0.20±0.05^{Aa}	*0.20±0.05^{Aa}
MODERATE SHADE	*H. courbaril*	0.84±0.02^{Aa}	*0.67±0.02^{Aa}	0.31±0.01^{ABb}	*0.28±0.01^{ABb}	*0.26±0.01^{ABb}	0.63±0.01^{ABb}	0.63±0.03^{Cb}	0.63±0.03^{Cab}	0.05±0.01^{Aa}	*0.10±0.04^{Aa}	*0.11±0.04^{Aa}
	C. guianensis	0.77±0.06^{Aa}	*0.65±0.06^{Aa}	0.34±0.04^{ABa}	*0.26±0.03^{ABCab}	*0.25±0.03^{ABa}	0.65±0.04^{Aa}	0.68±0.03^{ABb}	0.68±0.02^{ABb}	0.01±0.01^{Ba}	*0.06±0.01^{Aa}	*0.07±0.02^{Aa}
	H. brasiliensis	0.81±0.03^{Aa}	*0.68±0.03^{Aa}	0.37±0.04^{Aa}	*0.29±0.02^{Aa}	*0.28±0.02^{Aa}	0.59±0.03^{ABa}	0.62±0.00^{Cb}	0.63±0.01^{Cb}	0.04±0.01^{ABa}	*0.09±0.02^{Aa}	*0.09±0.02^{Aa}
	T. serratifolia	0.81±0.04^{Aa}	*0.69±0.04^{Aa}	0.28±0.01^{Bb}	*0.23±0.02^{BCb}	*0.23±0.01^{BCb}	0.67±0.01^{Aa}	0.67±0.01^{ABCa}	0.67±0.02^{BCb}	0.05±0.02^{Aa}	*0.10±0.02^{Aa}	*0.11±0.02^{Aa}
	B. grossularioides	0.75±0.10^{Aa}	*0.67±0.10^{Aa}	0.28±0.04^{Bb}	*0.22±0.02^{Cb}	*0.21±0.02^{Cb}	0.69±0.04^{Aa}	0.71±0.02^{Aa}	0.72±0.02^{Aa}	0.04±0.01^{ABb}	*0.07±0.01^{Ab}	*0.08±0.01^{Ab}
	O. pyramidale	0.82±0.02^{Aa}	*0.63±0.02^{Aa}	0.30±0.03^{Ba}	*0.24±0.02^{BCa}	*0.23±0.02^{BCa}	0.65±0.04^{ABa}	0.66±0.03^{BCa}	0.67±0.03^{BCa}	0.05±0.02^{Aa}	*0.10±0.01^{Ab}	*0.10±0.01^{Ab}
DEEP SHADE	*H. courbaril*	0.83±0.04^{Aa}	*0.66±0.04^{Aa}	0.42±0.03^{Aa}	*0.33±0.03^{Aa}	*0.31±0.03^{Aa}	0.57±0.03^{Ac}	*0.63±0.02^{Ab}	*0.64±0.02^{ABb}	0.01±0.01^{Bc}	*0.04±0.02^{Bb}	*0.05±0.01^{Bb}
	C. guianensis	0.81±0.05^{Aa}	*0.64±0.05^{Aa}	0.38±0.03^{Aa}	*0.29±0.01^{Aa}	*0.27±0.01^{Aa}	0.61±0.02^{Aa}	*0.66 ±0.01^{Ab}	*0.68±0.00^{Ab}	0.01±0.01^{Ba}	*0.05±0.01^{ABa}	*0.05±0.01^{Ba}
	H. brasiliensis	0.83±0.02^{Aa}	*0.65±0.02^{Aa}	0.45±0.11^{Aa}	0.31±0.05^{Aa}	*0.29±0.04^{Aa}	0.52±0.11^{Aa}	0.61±0.05^{Ab}	0.62±0.03^{Bb}	0.03±0.01^{ABa}	*0.08±0.01^{Aa}	*0.09±0.02^{Aa}
	T. serratifolia	0.85±0.05^{Aa}	*0.63±0.05^{Ab}	0.44±0.03^{Aa}	*0.32±0.02^{Aa}	*0.30±0.02^{Aa}	0.54±0.03^{Ab}	*0.62±0.02^{Ab}	*0.64±0.01^{ABc}	0.02±0.00^{ABb}	*0.06±0.01^{ABb}	*0.06±0.01^{ABb}
	B. grossularioides	0.86±0.04^{Aa}	*0.68±0.04^{Aa}	0.41±0.03^{Aa}	*0.30±0.03^{Aa}	*0.28±0.02^{Aa}	0.55±0.04^{Ab}	*0.63±0.04^{Ab}	*0.64±0.04^{ABb}	0.04±0.01^{Ab}	*0.07±0.02^{Ab}	*0.08±0.01^{ABb}
	O. pyramidale											

Mean ± standard deviation (n=4) follow in lines for same capital letter to different species in same environment and lower case to same species in different environment are equal by Tukey test ($p<0.05$). asterisks represent differences for the first measurement.

3.5 Relationships among leaf gas exchange traits

Considering the intraspecific and interspecific variations, we used PCA to investigate the associations among 34 leaf traits. The PCA explained 47.3% of the variation in leaf photosynthetic characteristics. The first axis contributed 32.3% of the power of explanation and with it ($r > 0.6$) A_{max}, LSP, ETR, R_P, E, ϕPSII, g_s, g_m, Vc_{max}, J_{max}, P_r, P_b, P_s, EUN and EUMn are strongly correlated. Moderate correlations ($0.3 < r < 0.6$) with this axis were observed with LCP, SLA, SL, ML and efficiency in the use of Ca, K, Fe, Zn and with EI (%). Axis 2 contains 15% of the variation and presented strong correlation ($0.6 < r < 0.9$) with R_d, SLA, LMA, ML and EUP. Among those with no correlation with axis 1, WUE, BL and EUMg showed moderate correlation with axis 2 (Figure 6). The distribution of the individuals in PCA demonstrates the greater photosynthetic potential of pioneer species in FS given its proximity to variables related to acquisitive strategies regarding resources use. This potential decreased along with irradiance, so the aggregate distribution in MS, including the non-pioneer in FS, showed the increase in potential of late-successionals and under-utilization of photosynthetic capacity of pioneer species in moderate irradiance environments. As in MS, the lower photosynthetic rates in DS induced aggregation of the individuals submitted to low irradiance, for which there was a notable investment in strategies that maximize the capture of the limiting resource, with an increase of P_l, SLA and EI (%).

Considering the variables related to A_{max} in each environment, there was a very strong positive correlation ($r = 0.96$) with ϕPSII and strong positive correlations ($0.6 < r < 0.9$) with ETR, R_P, E, Vc_{max}, J_{max}, N investments in rubisco (P_r) and in ETC (P_b), EUCa, EUN, EUFe and EUMn. With g_s and g_m, the correlations were also positive, however moderated. N investments in structural components (P_s) and stomatal limitations (SL) were negatively and strongly correlated with A_{max}.

For MS, as in FS, there was very strong correlation with ϕPSII. The strong correlations occurred with ETR, R_P, E, g_s and J_{max}. The moderated correlations occurred with LSP, Vc_{max}, EUCa, EUP, EUFe and EI (%). In this environment, unlike FS, there was no influence of g_m, partition of N, or any of the limitations on A_{max}.

Contrary to FS and MS, strong positive A_{max} correlations were found with EUMn and EUN, followed by ϕPSII, ETR, LSP, R_P, P_l and E. Moderate and positive correlations were found with LCP and J_{max}, while moderate negative correlations were found with P_s and EI (%).

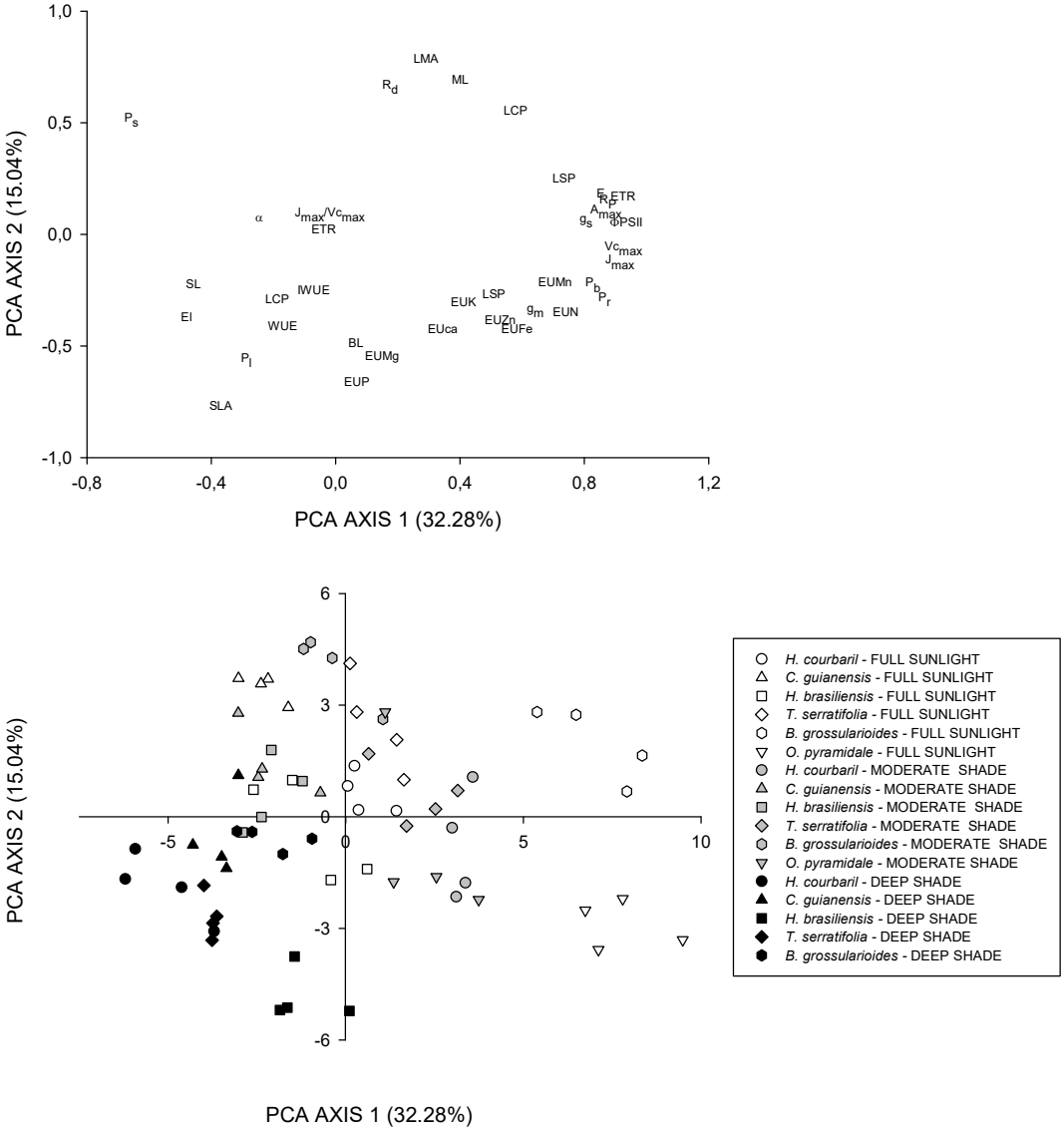

Figure 6: Principal component analysis of 34 photosynthetic leaf traits of saplings of six tree species subjected to different light environments.

4 **DISCUSSION**

4.1 Light and CO_2 curve responses

A_{max} values found in this study are in agreement with those observed in other research involving tropical tree species, especially those regarding the higher photosynthetic rates of pioneer species in high irradiance environments (Dias and Marenco, 2007; Gonçalves et al., 2012; Cunha et al., 2016; Guimarães et al., 2018). Except for *C. guianensis* and *H. brasiliensis*, in all other species studied, irrespective of the successional group, A_{max} decreased almost 50% in DS. For the aforementioned two species, the photosynthetic rates did not change between environments, suggesting lower modulation capacity of this process as a result of irradiance.

The flexibility of regulating photosynthetic capacity may be advantageous for plants such as *B. grossularioides* and *O. pyramidale*, growing in clearings and forest borders, since the increase of the density of vegetation occurs in the environment, thus these plants can experience different degrees of shading. In this respect, it is worth mentioning the greater adaptability to shade environment as shown by *B. grossularioides* in comparison to *O. pyramidale*, whose low photosynthetic flexibility may have been determinant for the failure of their establishment in DS.

These results corroborate with the evidence that the photosynthetic plasticity for acclimation to irradiance is not directly associated to the successional group in which the species are inserted (Rozendaal et al., 2006; DosAnjos et al., 2015).

Water vapor and CO_2 flows follow in opposite directions, but share a common path through stomatal pores on the leaf surface, so a trade-off between transpiration costs and CO_2 assimilation is implicitly unavoidable (Martins et al., 2014). As a consequence, the values of E and g_s tend to present a strong positive correlation ($r = 0.74$ $p < 0.001$) and in any case, higher g_s and E were shown by plants with higher A_{max}, that is, generally in FS. Although there was an increase in water loss due to transpiration, none of the plants exhibited differences between WUE and IWUE in the higher and lower irradiance environments, similar to that observed by Marenco et al. (2001) and Gonçalves et al. (2012). It should be noted that, during the experiment, there was daily control of irrigation, so, the stomatal behavior in this study was an exclusive and individual consequence of species' responses to irradiance treatment and not due to any water limitations on the substrate.

Just as g_s, g_m also undergoes influence of the water status of the plant and alterations in its behavior can cause restrictions in transference of CO_2 from intracellular space to carboxylation sites in stroma, it is able to imply alterations in photosynthesis (Tezara et al., 2011). Positive correlations between g_s-A_{max} and g_m-A_{max} in FS indicate that the diffusion of external air to carboxylation sites affected photosynthesis in this environment. Higher g_m in sunlit leaves have been frequently reported, similar to what was observed here, except for *H. brasiliensis* and *C. guianensis*, whose photosynthetic rates did not differ between environments, highlighting the contribution of g_m to A_{max} potentiation under high irradiance. The variations in g_m are still under-

explored, but it is believed that it may be partly explained by the morphoanatomic differences (LMA) between leaves (Warren, 2008; Campany et al., 2016).

Other characteristics related to the photosynthetic yield are the light compensation (LCP) and saturation (LSP) points. Increasing LSP means photochemical enhancement of light at higher intensity for longer periods of time, which may be particularly interesting for high irradiance plants, since the absorption of light above the photochemical utilization capacity may lead to the formation of reactive oxygen species (ROS), thus increasing the probability of oxidative damages in photosynthetic apparatus. In accordance with what was found in other studies, LSP was higher in environments with higher irradiance. LCP, on the other hand, becomes advantageous when reduced, especially under low irradiance. Modulations in LCP are always accompanied by changes in R_d, since the decrease in respiratory rates represents a lower maintenance cost for metabolism, which promotes a positive CO_2 balance in lower irradiance, even with lower photosynthetic rates (Valladares and Niinemets, 2008; Sterck et al., 2013; DosAnjos et al., 2015).

The greater availability of light in FS certainly induced a greater flow of electrons through the transport chain in order to supply the demands of ATP and NADPH to be consumed in photochemical processes, which raises the ETR and R_P values in this environment (Santos Junior et al., 2006). In view of the magnitude of the difference in ETR between pioneers and non-pioneers, the higher photochemical efficiency of this group under high irradiance is clear. As the irradiance decreases, the difference between pioneers and non-pioneers also decreases. Regarding R_P, in this study the photorespiratory cost represented between 31%-57% of A_{max} in FS and 27%-34% in MS and DS. Data founded in literature show that photorespiratory rate can vary between 10-38% of A_{max} (Carneiro et al., 2015; Gonçalves et al., 2012; Marenco et al., 2001; Martins et al., 2014). In *C. guianensis* and *H. brasiliensis,* the photorespiratory costs reached 45% and 57% in FS, respectively, which may compromise, at least partially, the photosynthetic performance under high irradiance. However, while high R_P can be considered harmful to biomass accumulation under higher irradiance, this process plays an important photoprotective role in plants under stress conditions, dissipating excess energy and reducing the risk of more severe photoinhibitory damages (Bai et al., 2008; Voss et al., 2013).

V_{cmax} has been identified as an important predictor of the ability to adjust photosynthetic machinery (Niinemets et al., 2007; DosAnjos et al., 2015), and the close correlation with J_{max} makes them key factors for the study of acclimation to irradiance. In general, Vc_{max} and J_{max} were strongly related to A_{max} independent of the environment ($r = 0.765$ $p < 0.001$ and $r = 0.769$ $p < 0.001$, respectively), as observed in other studies (Rodríguez-Calcerrada et al., 2008; Dalmagro et al., 2013). The values observed for both reveal the highest A_{max} for pioneer species in FS, for *H. courbaril* in MS and reveal the similarity among the environments for *C. guianensis* and *H. brasiliensis*. The analysis of these variables on mass basis reveals different patterns of resource allocation to increase efficiency in different situations. Contrary to the base area, both mass bases increased with decreasing irradiance, however, the proportional increase in DS was higher for

J_{max} than V_{cmax}, suggesting a greater investment in the photochemical stage in detriment to the biochemical stage of photosynthesis.

The J_{max}/V_{cmax} ratio provides an indication of the nitrogen partition between thylakoids (N associated with pigments in photochemical stage) and soluble proteins (N associated with Calvin Cycle proteins). It was observed that plants that suffered the highest proportional reduction of A_{max} in DS exhibited higher J_{max}/V_{cmax}. Walker et al. (2014) suggests the existence of a trade-off between J_{max} and Vc_{max} in plants under light-limited conditions, which corroborates the results found here.

4.2 Photosynthetic limitations

In general, diffusive limitations (SL+ML) imposed the greatest restrictions on photosynthesis in all environments for all species, regardless of the successional group. The ML may assume similar magnitudes to SL and, while aggregated, are generally larger than the BL under stress conditions (Flexas et al., 2012; Xiong et al., 2018). Considering the morphological changes that occur due to irradiance (higher LMA), it is probable that the thickening of the parenchyma associated with the reduction of stomatal opening has restricted the CO_2 influx from the atmosphere to the leaf, thus constituting a major obstacle to diffusion of carbon in mesophilic tissues, increasing ML in FS and MS and contributing to its reduction in DS.

The ratio of SL + ML and BL was more balanced in DS for intermediate species and for the late successional *C. guianensis*. In this sense, our attention is drawn to the difference between the pioneer species, where *B. grossularioides* suffered in FS and MS less BL than O*. pyramidale*, which in turn was the most limited among all the species in these two environments, suggesting that carbon assimilation strategies in limiting environments may be different among species of the same successional classification. This was expected for all species larger BL in DS, especially due to trade-off between J_{max} and V_{cmax}, however for *H. courbaril* (late successional) and *B. grossularioides* (pioneer) the opposite occurred, even with decreases of V_{cmax} to about 1/3 which were observed in FS. While analyzing g_s, these species showed a greater proportional reduction between FS and DS (almost 3 times for *H. courbaril* and 2 times for *B. grossularioides*), suggesting that the restriction of CO_2 at the carboxylation site due to diffusive limitations may have outweighed eventual problems with rubisco activity.

4.3 Partition of leaf nitrogen

The photosynthetic efficiency in nitrogen use is positively correlated with A_{max} in this study and the differentiated partition observed among the environments demonstrated its importance for the plants' acclimation to irradiance in accordance with Li et al. (2008) and Moon et al. (2015).

The high P_r of pioneer species in comparison to others, especially the late successional *C. guianensis*, was determinant for the higher V_{cmax} and A_{max} of these species in FS. For late sucessionals, P_s almost three times greater than the pioneers suggests both the construction of

more robust leaf structures, since species with a longer life cycle and conservative strategies tend to have longer leaves according to leaf economy spectrum (Takashima et al., 2004; Poorter et al., 2009; Reich, 2014), and as they also attempt to repair cell damage caused by high irradiance in FS (details in Chapter 3).

With the decrease in irradiance, we expect a reallocation of N invested in rubisco for components related to the photochemical phase in order to increase light absorption capacity (Niinemets et al., 1998), and indeed our results found this expectation. The ability of *B. grossularioides* to modulate the investments between FS and DS by reducing P_r and practically doubling P_l and P_b justifies the higher EUN and A_{max} of this species in DS. For *H. brasiliensis* the reallocation of N between P_s e P_l was due to the reduction in almost 2/3 of P_s observed in FS. This specie exhibited the highest P_r and P_b in DS, which may explain the higher V_{cmax} and J_{max} exhibited in this environment, however, without increases in A_{max}.

4.4 Photosynthetic induction curve

The start of induction curves shows well-marked differences in the velocity of adjustment of photosynthetic parameters studied regarding the pioneer and late-successional species, with the first group exhibiting more than twice the A_i, g_{si} and ETR_i in comparison to the others, besides reaching saturation in almost half the time. The values for gs_i found for late-successionals are similar those reported by Bai et al. (2008) and Urban et al. (2007) for shade-tolerant species.

The sigmoidal behavior for late-successional species is typical in species with low g_{si} and to it can be attributed to the lower states of induction in the first minutes of the curve and the greater time to reach the photosynthetic saturation in all the environments (Valladares et al., 1997). After 5 minutes in saturating light, the late successional species in DS had a higher activation state than plants in FS, but for *B. grossularioides* (the only surviving pioneer) the DS plants only showed a higher activation state compared to FS after 25 minutes, revealing the greater capacity of shade-tolerant species to respond promptly to light stimuli after dark periods.

Regarding the limitations occurred during induction, in DS, SL exceeded BL only in *H. courbaril* and *C. guianensis*, which occurred before 20 min, due the greater proportional increase of A in relation to g_s. Since shade-tolerant species are generally more sensitive to dryness than pioneers (Niinemets et al., 1998), water loss as a result of increased transpiration induced the stomatal closure, restricting photosynthesis.

In environments with higher irradiance, BL was always higher than SL, and BL is mainly determinate for the activity/concentration of the enzyme Rubisco activase and the concentration of Calvin cycle metabolic intermediates (Parry et al., 2008). Shade species generally have higher proportions of Rubisco activase than Rubisco, allowing for faster activation of the enzyme (Sage et al., 2002).

According to the observed results, as excepted for *B. grossularioides*, the plants in DS however need more time to raise the state of Rubisco activation to maximum which shows a lower loss of photosynthetic induction after the 15 min in the dark, probably due the greater proportion between Rubisco activase and Rubisco, which allows them better use of sunflecks, a fundamental characteristic for plants that inhabit the understory. It should be noted that, in FS and MS, the pioneer *O. pyramidale* displayed the greatest loss of activation among the studied species, which may have been another contributing factor for the low photosynthetic plasticity that prevented its development in the shade.

Concerning the fluorescence parameters, only *C. guianensis* in FS exhibited signs of photoinhibition at the start, denoted by a F_v/F_{mi} of less than 0.75 (Krause et al., 2001). For DS plants, the data show no signs of photoinhibition at the end of the induction period, an increase of the dissipation capacity of excess energy (ϕNPQ) and an increase photochemical yield (ϕPSII), which indicates favorable conditions for the use of sunflecks without any damage to photosynthetic machinery (Martins et al., 2013).

4.5 Relationships among leaf gas exchange traits

The arrangement of *B. grossularioides* and *O. pyramidale* on the positive portion of axis 1 (Figure 6) denotes the greater photosynthetic potential of these species in FS. The difference between them was the lower LMA, the most pronounced BL in *O. pyramidale* and the contributions of g_m to reduce ML in this same species, highlighting the importance of this parameter in studies on acclimation to irradiance (Flexas et al., 2012). In FS, the non-pioneer species had the majority of individuals located in the negative part of axis 1, revealing the greater SL and the high P_l. Despite the grouping between non-pioneers in FS and MS, it is still possible to observe the distance of *C. guianensis* from the others, showing a lower tolerance to high irradiance.

H. courbaril and *T. serratifolia* exhibited similar behavior between FS and MS, displaying lower performance in DS, despite belonging to different succession groups. In the same way, *C. guianensis* and *H. brasiliensis* were similar among the three environments for A_{max} and other photosynthetic parameters.

In this study the differentiation of A_{max} between the higher irradiance environments and DS appears occurs under a strong influence of nitrogen use. The lowest variations in EUN between environments were observed for *C. guianensis* and *H. brasiliensis*, which may be the cause of similar photosynthetic rates between FS and DS. The partition of this element among photosynthetic components was also more stronger than observed by Katahata et al. (2007) and Niinemets et al. (1998), especially regarding the modulation of P_r to overcome biochemical limitations in FS, and of P_l to increase the light capture efficiency in DS.

Higher A_{max} levels were always positively related to P_r and Vc_{max} in FS, while in DS they did not have a significant effect on A_{max}. Considering the negative correlations between EI (%) and Vc_{max} and P_r in DS (less A_{max}) and FS (greater A_{max}), it is possible conclude that, although it is an important strategy for the use of *sunflecks* in low irradiance environments, maintenance of a high level of rubisco activation (probably induced by the high amount of the rubisco-activase enzyme) was not sufficient to compensate the smaller amount of the carboxylation enzyme itself, which impaired the carbon fixation in Calvin cycle.

It should also be noted that in low light environments there is a major need to enlarge the LHC, directing more nitrogen to this compartment than when in the environments with higher irradiance, thus contributing to the lower availability of N for rubisco synthesis in these environments. Therefore, it is possible to observe that the plants studied, when kept in an environment with irradiance for a longer time close to LCP, had photosynthetic disturbances that were more difficult to balance than those maintained above LSP, even when under photoinhibitory conditions.

CONCLUSIONS

The photosynthetic behavior of the species studied varied irrespectively of their successional group, which confirms that the photosynthetic plasticity is not necessarily associated to ecological groups, and is influenced to a greater degree by the individual characteristics of the species in acclimation to the environment.

The photosynthetic limitations were predominantly diffusive and the mesophilic limitation was similar to stomatal in the species grown in environments with greater irradiance, and these had less magnitude under restriction of this resource, where biochemical limitations overcome them.

Highest N allocation in Rubisco was the main cause of high photosynthetic rates of pioneer species under high irradiance, while non-pioneer species prioritize investments in structural components, probably seeking greater leaf longevity, but reducing the short-term potential of photosynthetic processes. The species with lower flexibility in the N partition showed minimal variations in the photosynthetic rates, regardless of changes in environment. Thus, leaf nitrogen partition between photosynthetic and structural compounds is more determinant for photosynthesis than its own content. The capacity to modulate N allocation is determinant for the increase or decrease of photosynthesis in response to changes in light conditions.

Late successional species maintained a higher photosynthetic induction state under low irradiance, probably due to the greater proportion of Rubisco activase in comparison to pioneers, conferring greater ability to take advantage of sunflecks. However, despite being an efficient short-term strategy, it imposes biochemical limitations in less time, restricting the photosynthetic capacity of the plant.

Finally, all species were able to tolerate high irradiance, but one of the pioneer species did not survive the shading, suggesting that acclimation at low irradiance is more difficult to tolerate than high irradiance.

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CHAPTER 3

PHOTOCHEMICAL EFFICIENCY AND ANTIOXIDATIVE METABOLISM OF SIX TREE SPECIES IN RESPONSE TO IRRADIANCE

PHOTOCHEMICAL EFFICIENCY AND ANTIOXIDATIVE METABOLISM OF SIX TREE SPECIES IN RESPONSE TO IRRADIANCE

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ABSTRACT

Light provides the energy necessary for photosynthesis, thus determining the distribution, survival, growth and development of plants. Thus, plants, especially trees, should be able to respond positively to variations in the availability of this resource in the natural environment, given the great heterogeneity with which light presents itself. The balance between efficiency of absorption and use of light energy is fundamental to supply the energetic demands of the metabolism and to avoid photoinhibitory damage by excessive irradiance. In this study, we investigated the effects of different irradiance levels on the photosynthetic apparatus of six tree species submitted to three different environments (full sunlight-FS, moderate shade-MS and deep shade-DS). We evaluated changes in leaf pigment content, photochemical yield and efficiency in the dissipation of excess energy by chlorophyll *a* fluorescence, the functioning of the antioxidant system and lipid peroxidation. The 29 photochemical and enzymatic variables were correlated with each other and with the foliar nutrient contents. There was adjustment of the light harvest complex (LHC) in DS plants, with increased concentrations of foliar pigments. In general, the pioneer species exhibited more efficient photochemical yield and antioxidant enzymatic system. The plants in FS showed higher intensity of lipid peroxidation, with SOD having a prominent role in the antioxidant system. At lower irradiance the enzymatic activity was reduced and the photochemical efficiency was the best way to reduce oxidative damages. As for the nutrients, P was highly related to the photochemical yield and the N modulation amplified the LHC in DS in detriment to the antioxidant system. In FS, the opposite was observed. Despite the evidence of cell damage, most species exhibited the ability to adjust to high irradiance. Only *C. guianensis* (late successional) exhibited photoinhibitory damage in FS and O. *pyramidale* (pioneer) did not survive the shading in DS.

Key-words: Chlorophyll fluorescence, ROS, nutritional status and acclimation.

Chapter formatted according to *Environmental and Experimental Botany* journal instructions

1. INTRODUCTION

As a primary source of energy, light exerts influence on the physiological processes, since it is primordial for energy flux in biological systems and highly determinant for plant growth, development, morphogenesis and other physiological responses. However, the heterogeneous form it presents in nature requires that the plants exhibit great flexibility due to spatial and seasonal changes in the availability of this resource (Baird et al., 2017).

The ability of a genotype to express different phenotypes in response to environmental conditions is known as phenotypic plasticity, which is a key feature for acclimation (Valladares et al., 2016). When exposed to changes in irradiance conditions, plants respond with changes in leaf characteristics and physiological functions that represent important aspects to be considered during the acclimation process. All these modifications occur in order to maximize the efficient use of the light energy which is absorbed in the photosynthetic process and minimize the occurrence of photoinhibition. This term describes a reversible or irreversible decline of photosynthetic activity when light energy is absorbed beyond the photosynthetic capacity of the plant (Walters, 2005; Dietz, 2015; Lestari and Nichols, 2017).

One way to reduce the effects of intense irradiance is to dissipate excess energy in non-photochemical processes, such as dissipation in the form of heat, via the xanthophyll cycle, and in the form of fluorescence. Many studies have been carried out using the measurement of chlorophyll *a* fluorescence in order to evaluate photoinhibitory damage caused by excess energy and all point to photosystem II (PSII) as the first target of the photoinhibitory damages (Tikkanen et al., 2012; Mathur et al., 2014).

The proteins of PSII are very susceptible to damage caused by ROS, which are partially reactive forms of highly reactive molecular oxygen, and can lead to oxidation of important cellular components such as cell membranes, lipids and even the genetic material of the cell. To avoid oxidative damage, plants have enzymatic and non-enzymatic systems for removal of ROS, which may be directly related to the tolerance of plants to the stress situation (Derks et al., 2015; Li et al., 2018).

The antioxidant enzyme SOD is considered the first line of defense in the fight against ROS, transmuting $O_2\bullet^-$ to form H_2O_2. In addition, the CAT, APX and POX enzymes complement the EROS elimination process by transforming H_2O_2 into water and molecular oxygen (Barbosa et al., 2014; Wu et al., 2015).

All this dynamic of the light regime imposes a series of difficulties for the survival of the plants in natural environment or even in conditions of planting. In this scenario, the investigation of the physiological performance of young plants in relation to the incidence of light is justified, given not only the heterogeneity with which this resource presents itself in a natural environment, but also because of its strong influence on plant metabolism.

In this context, the objective of this study was to investigate the photochemical performance and behavior of the antioxidative system of species from different successional groups to the contrasting irradiance to understand how both could contribute to the control of possible oxidative damages due to changes in the light environment.

2. MATERIAL AND METHODS

2.1 Plant material and growth conditions

The study was conducted at the National Research Institute for the Amazon - INPA (Manaus, Amazonas - Brazil). Saplings of 6 native Amazonian species belonging to three distinct succession groups (Table 1) were cultivated in the nursery and when they reached 9 months of age were transplanted into plastic pots containing12 liters of substrate (regional latosoil collected in the native forest with organic matter – see supplementary material for nutritional characteristics). At 12-14 months of age, one part of the group was transferred to 2 different irradiance environments and one part was kept in the nursery (4 individuals per species in each treatment). Incident photosynthetic radiation was monitored with a line quantum sensor (model LI-191, LI-COR Inc., Lincoln, Nebraska, USA) for 7 sunny days.

Table1: List of studied species with scientific name, family and successional group as described in literature.

Specie	Family	Sucessional group	Reference
Hymenea courbaril	Fabaceae	Late-sucessional	Souza et al. (2009)
Carapa guianensis	Meliaceae	Late-sucessional	Vinson et al. (2005)
Hevea brasiliensis	Euphorbiaceae	Mid-sucessional	Amaral et al. (2009)
Tabebuia serratifolia	Bignoniaceae	Mid-sucessional	Gualberto et al.(2014)
Bellucia grossularioides	Melastomataceae	Pionner	Bentos et al. (2017)
Ochroma pyramidale	Malvaceae	Pionner	Slot and Winter (2018)

The light treatments (Table 2) consisted of full sunlight (FS) (100 % of solar irradiance, simulating a forest clearing), artificial moderate shade (MS) provided by shade cloths reducing direct, incident solar radiation (simulating an understory light environment with partial canopy openness) and natural deep shade (DS) with natural shade provided by adult tree canopies (simulating an understory light environment). The plants were subjected to these treatments during 180 days.

Table 2: Daily PAR average, maximum PAR observed, average percentage of full sunlight under three light environments.

Specie	Full Sunlight (FS)	Moderate Shade (MS)	Deep Shade (DS)
PAR average	1027.51±10.49	362.50 ± 9.37	47.95 ± 7.98
Maximum PAR	1866.17 (13:00)	795.61 (12:00)	74.50 (14:00h)
% PAR	100	35.23	4.66

2.2 Chloroplastidic pigments content

Fully expanded and healthy leaves were collected, covered with aluminum paper immediately frozen in liquid nitrogen and stored at -80°C until the moment of analysis.

The analysis was performed using 0.1 g of fresh material ground in 10 ml of 80% acetone with magnesium carbonate ($MgCO_3$) and 10 ml of 100% acetone added immediately following the initial grinding step. The suspension was filtered, and the absorbance was read at 663 nm (Chl a), 645 nm (Chl b) and 480 nm (Car) using a spectrophotometer (Ultrospec 2100 pro UV/visible, Amersham Biosciences, Cambridge, UK) (Lichtenthaler and Wellburn, 1983). The chlorophylls *a* (Chl *a*) and *b* (Chl *b*) and carotenoids (Car) contents were calculate using equations described by Hendry and Price (1993), as show bellow:

$$\text{chl}a\ (\mu\text{mol g}^{-1}\text{or } \mu\text{mol m}^{-2}) = \frac{(12.7 \times A663 - 2.69 \times A645) \times 1.119 \times V}{1000 \times \text{area unit (m}^2\text{) or mass (g)}}$$

$$\text{chl}b\ (\mu\text{mol g}^{-1}\text{or } \mu\text{mol m}^{-2}) = \frac{(22.9 \times A645 - 4.68 \times A663) \times 1.102 \times V}{1000 \times \text{area unit (m}^2\text{) or mass (g)}}$$

$$\text{Car}\ (\mu\text{mol g}^{-1}\text{or } \mu\text{mol m}^{-2}) = \frac{(A480 + 0{,}114 \times A663 - 0.638 \times A645) \times V \times 1000}{112{,}5 \times \text{area unit (m}^2\text{) or mass (g)}}$$

A represents the absorbance at the indicated wavelength, V is the final volume of the chlorophyll-acetone extract (ml). The total chlorophyll (chl_{tot}) contents is the sum of Chl *a* and Chl *b*. For their ecophysiological implications, we calculate ratios chl*a*/chll b and total chl_{tot}/Car contents.

2.3 Chlorophyll fluorescence parameters

Leaf gas exchange and chlorophyll *a* fluorescence were measured simultaneously using an open-flow infrared gas exchange analyzer system equipped with a leaf chamber fluorometer (LI-6400XT, Li-Cor, Lincoln, NE, USA). The measures have been taken between 7:00 a.m. and 1:00 p.m.

Fully expanded and healthy leaves were sampled and acclimated to darkness for 30 min and a weak modulated measuring beam (0.03 µmol m^{-2} s^{-1}) was applied to obtain the minimal fluorescence (F_0). The maximum fluorescence emissions (F_m) were measured after applying a saturating white light pulse of 8,000 µmol m^{-2} s^{-1} for 0.8 s.

The leaf was enclosed in the gas exchange system and left under baseline conditions until net assimilation (A), stomatal conductance (g_s) and internalCO_2 concentration (C_i) stabilized. The baseline conditions inside the leaf cuvette included CO_2 concentration (Ca) 400 µmol mol^{-1}, relative humidity around 60% and 30 ºC temperature and PPDF of 1500 µmol m^{-2} s^{-1}. After the acclimation period, the fluorescence signals F_s (steady-state fluorescence under actinic illumination of 1,500 µmol m^{-2} s^{-1}), F_m' (maximum fluorescence during a light-saturating pulse of 8,000 µmol m^{-2} s^{-1}) and F_0' (light-adapted minimal fluorescence, obtained using a weak far-red illumination) were measured simultaneously with photosynthetic responses to irradiance (A/PPDF

curves) in 14 photosynthetic photon flux density (PPFD) levels (0, 10, 25, 50, 75, 100, 150, 200, 300, 500, 750, 1000, 1500 and 2000 µmol m^{-2} s^{-1} in decreasing order).

The calculated parameters are show in Table 1:

Table 3: Fluorescence parameters calculated from saturating pulse analysis:

Fluorescence parameter	Name and Physiological interpretation
$F_v/F_m = (F_m - F_0)/F_m$	Estimated maximum quantum efficiency (yield) of PSII photochemistry[1]
$F_v'/F_m' = (F_m' - F_0')/F_m'$	Maximum efficiency of PSII photochemistry in the light, if all centers were open[2].
$\phi PSII = (F_m' - F')/F_m'$	Estimated effective quantum yield (efficiency) of PSII photochemistry at incident PAR[3]
$ETR = \alpha\beta \times PAR \times \phi PSII$	Electron transport rate in PSII at incident PAR. ($\alpha\beta$ was determined from curves obtained under non-photorespiratory conditions in an atmosphere containing less than 1% O_2)[3,4]
$NPQ = (Fm - F_m'/F_m')$	Non-photochemical quenching[5,6]
$qP = (F_m' - F_s)/(F_m' - F_0')$	Coefficient of photochemical quenching based on the ‘‘puddle’’ model (i.e., unconnected PSII units)[7]
$qL = qP \times (F_0'/F_s)$	Coefficient of photochemical quenching based on the ‘‘lake’’ model (i.e., fully connected PSII units)[2]
$\phi NO = 1/[NPQ + 1 + qL(F_m/F_0 - 1)]$	Quantum yield of non-regulated energy dissipation in PSII[2]
$\phi NPQ = 1 - \phi PSII - \phi NO$	Quantum yield for dissipation by down-regulation[2]

[1]Krause and Weiss (1991), [2]Kramer et al. (2004), [3]Genty et al. (1989), [4]Yin et al. (2009), [5]Bilger and Björkman (1990), [6]Walters and Horton (1991), [7]Schreiber et al. (1986).

Nonlinear regression models were fitted to describe the variations in photochemical and non-photochemical yields with PPF for each sapling (Rodríguez-Calcerrada et al., 2008).

2.4 Extraction of soluble protein and measurement of enzyme activities

Leaves in same conditions as the pigments analysis were collected at 1:00 p.m and immediately frozen in liquid nitrogen. For the analysis of enzyme activity, leaves samples were ground to a fine powder and homogenized in 100 mM potassium phosphate buffer (pH 6.8) containing 1mM EDTA, PMSF 1 mM and 1% (w/v) soluble PVPP. The homogenate was centrifuged at 15000×g for 20 min. The supernatant was collected and aliquots were used for enzyme analysis. All extraction procedures were performed at 4°C.

The activity of Catalase (CAT; EC 1.11.1.6) was measured spectrophotometrically at 270 nm by determining the rate of H_2O_2 conversion to O_2 (Azevedo et al., 1998).The activity of ascorbate peroxidase (APX; EC 1.11.1.11) was measured as a decrease in absorbance at 290 nm, which results from ascorbate oxidation (Nakano and Asada, 1981). The activity of peroxidases (POX; EC 1.11.1.7) was measured as a decrease in absorbance at 420 nm, which results from purpurogallin formation (Kar and Mishraa, 1976) The superoxide dismutase (SOD; EC 1.15.1.1) was measured spectrophotometrically at 560 nm by detecting the inhibition of

nitroblue tetrazolium (NBT) reduction by SOD (Beauchamp and Fridovich, 1971). One unit of SOD was defined as the amount needed for 50% inhibition of NBT reduction (Giannopolitis and Ries, 1977). Total soluble protein was determined according Bradford (1976), using bovine serum albumin (BSA) as the standard.

2.5 Lipid peroxidation (Determination of malonaldehyde content)

The lipid peroxidation was estimated by formation of thiobarbituric acid (TBA) reactive substances (Cakmak and Horst, 1991). Absorbance was measured at 535 and 600 nm and malonaldehyde (MDA) concentrations were calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

2.6 Leaf phenols compounds

Total phenolic compounds were determined by Jennings (1981) and using the Folin-Ciocalteau reagent. The absorbance was measured at 725 nm and utilized tannic acid in the calibration curves.

2.7 Experimental design and statistical analysis

The experimental design was factorial completely randomized (6X3) with six species in three treatments. After the assumptions of normality and homoscedasticity were compiled, to analyze the differences between species and treatments, the appropriate analyses of variance and the averages were performed using the Tukey post hoc test. When appropriate, relationships between variables were tested by regression equations, using as criteria for adjustment: 1) significance of the adjusted regression; 2) significance of its coefficients and 3) higher coefficient of determination. The interrelationships among functional traits variables were assessed using the principal components analysis (PCA) ordination method.

All analysis were performed with Statistica 8.0 (Statsoft Inc. 2007), SPSS 23 (IBM Corp. 2015) and SigmaPlot 11 (Systat software, 2008). To determine the relationship between the different variable parameters, we calculated the Pearson's correlation coefficients. The correlation matrixes were prepared by R program (http://www.R-project.org). The values of the correlation coefficient varied between + 1 and −1. When the value is around + 1 or − 1, it indicates a close positive or negative relationship between the variables, respectively. As the correlation coefficient value approximates 0, the relationship between the two variables will become weaker.

3 RESULTS

3.1 *Chloroplastidic pigments content*

Plants in DS exhibited higher contents of Chl *a* and *b*, on area and mass basis, than FS plants (Table 4). In MS only *H. courbaril* and *T. serratifolia* matched the contents found in DS, which gave them greater photosynthetic advantage when compared with others in this environment (see chapter 2). This was repeated for the *Car* and Chl_{tot} concentrations in the area base, but in the mass base both total pigments and individual concentrations were higher in DS than in the other environments (Table 4).

The Chl *a:*Chl *b* ratio decreased with decreasing irradiance (higher in FS) while Chl_{tot}:*Car* showed opposite behavior (higher in DS). It is important to note that both are important physiological indicators of the adaptation of plants to light environments.

Table 4: ANOVA results for environments, species and interactions (environments x species) showing statistic F and p-value for chlorophylls *a, b* and total, carotenoids (mass and area basis), ratio chlorophyll a: chlorophyll b and ratio total chlorophylls:carotenoids.

Variable	Environment		Specie		Interaction	
	F	p	F	p	F	p
chl*a* (µ mol g^{-1})	176,85	<0,001	14,14	<0,001	4,58	<0,001
chl*b* (µ mol g^{-1})	250,79	<0,001	22,51	<0,001	16,47	<0,001
chl_{tot} (µ mol g^{-1})	207,46	<0,001	16,75	<0,001	7,45	<0,001
car (µ mol g^{-1})	151,59	<0,001	21,52	<0,001	8,77	<0,001
chl*a* (µ mol m^{-2})	77,33	<0,001	4,44	0,002	6,86	<0,001
chl*b* (µ mol m^{-2})	117,58	<0,001	8,05	<0,001	10,31	<0,001
chl_{tot} (µ mol m^{-2})	92,88	<0,001	5,43	<0,001	7,66	<0,001
car (µ mol m^{-2})	55,74	<0,001	7,18	<0,001	9,20	<0,001
chl*a:*chl*b*	44,84	<0,001	3,37	0,011	2,66	0,013
chl_{tot}:car	80,82	<0,001	5,46	<0,001	1,96	0,064
SPAD	281,71	<0,001	20,54	<0,001	18,63	<0,001

3.2 *Chlorophyll a fluorescence parameters*

With regard to the chlorophyll *a* fluorescence, only *C. guianensis* showed a F_v/F_m ratio lower than 0.75 and these values were found in FS, denoting higher susceptibility of these species to photoinhibition when under high irradiance. Except for these two, despite small differences between absolute values, other studied plants showed no signs of exacerbated photochemical limitations (Figure 1).

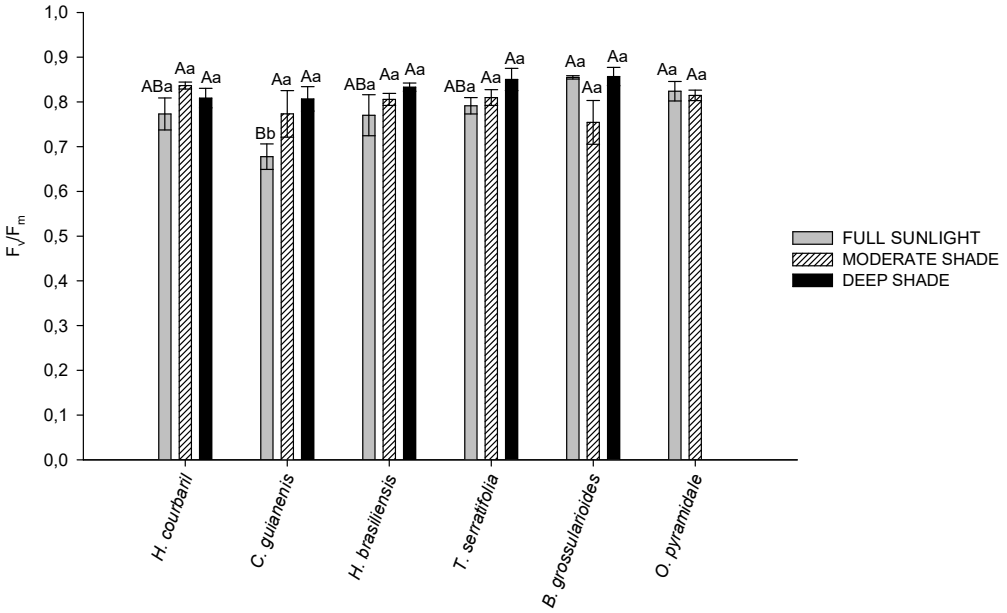

Figure1: Maximum quantum yield of PSII photochemistry of six tree species submitted to three different light environments. Same capital letters for different species in same environment and small case for same species in different environment are equal by Tukey test ($p < 0,05$). Vertical bars indicate the standard error (n=4).

In general, the ETR was higher in FS than in other environments. Under saturation light (1500 µmol m^{-2} s^{-1}), *B. grossularioides* and *O. pyramidale* exhibited the highest values, the first being 1.8 and 2.1 times greater than MS and DS and the second 1.5 times greater than MS, respectively (Figure 2 A1-A3). All individuals of *O. pyramidale* (pioneer) in DS were already dead at 97 days of experiment. In MS, the highest values observed were for *H. courbaril*, followed by *T. serratifolia*. *B. grossularioides* and *H. brasiliensis* displayed higher ETR in DS, however, while the first showed high performance in FS, the second had little difference between the environments.

In absolute terms, the fraction of electrons destined for carboxylation (ETR_C) was higher in FS plants than MS for *H. brasiliensis* (+11.1%), *B. grossularioides* (+41.2%) and *O. pyramidale* (+28.3%). The late-successional *H. courbaril* and *C. guianensis* showed higher ETR_C in DS than FS (+19.5% and +11.6%, respectively). On the other hand, FS plants proportionally exhibited the largest electron fractions for oxygenation (ETR_O), denoting the highest photorespiratory cost of the plants in this environment, as already discussed in Chapter 2 (Figure 3 B1-B3).

Higher F_v '/ F_m' values were observed for DS plants and lower FS plants (Figure 2 C1-C3). In FS, the highest value was observed in *H. courbaril* and the lowest in *C. guianensis* (-25.5%). In MS, *H. courbaril* values were also higher, but this time the species with lower values was *B. grossularioides* (-33.6%). *B. grossularioides* had highest yields in DS while *H. brasiliensis* had the lowest (-19.3%).

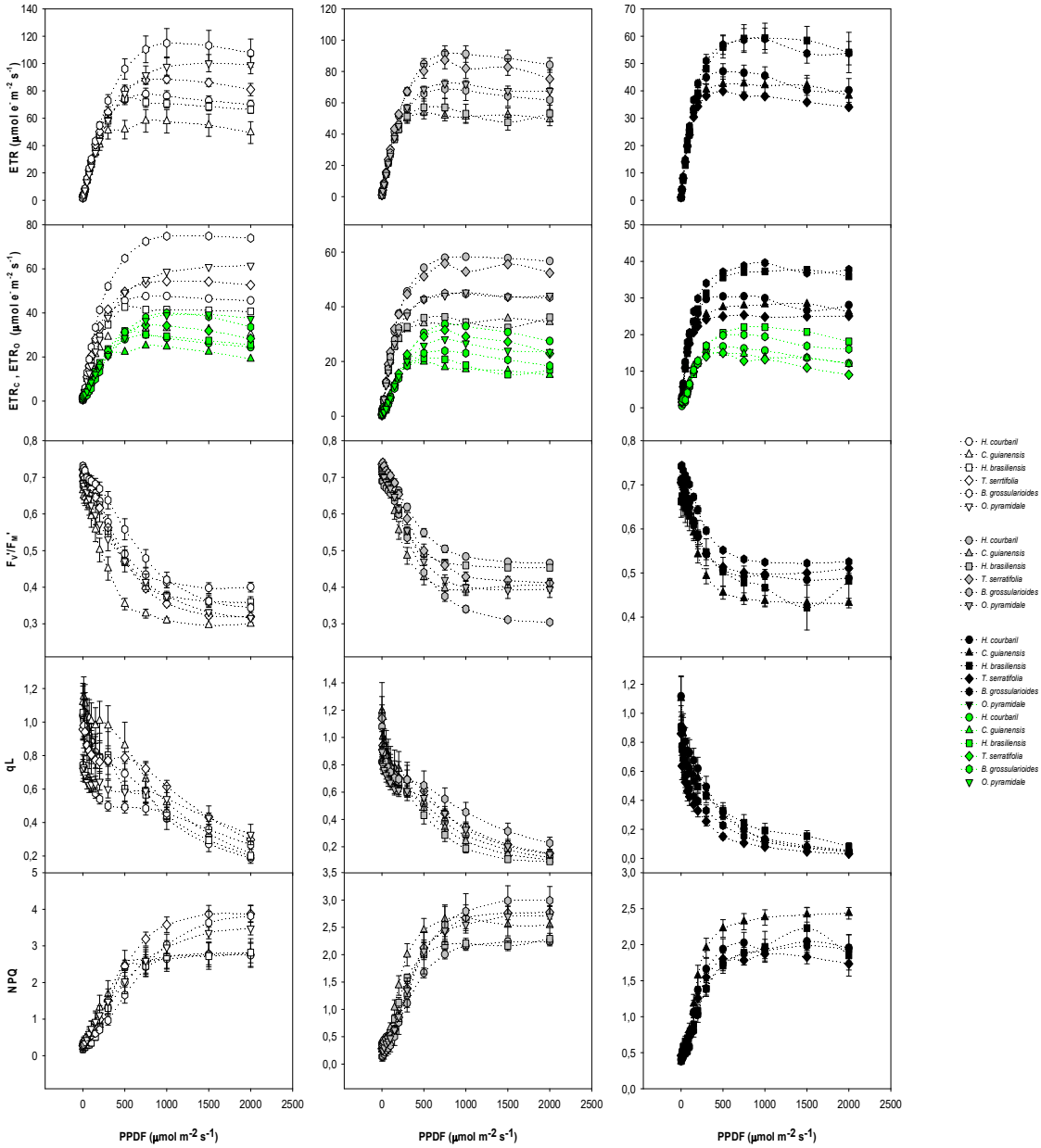

Figure 2: Fluorescence parameters in function of irradiance (PPDF): A) Total electrons transport rate (ETR), B) fraction of electrons destined for carboxylation (ETR_C) and oxygenation (ETR_O – green symbols), C) Maximum efficiency of PSII photochemistry in the light (F_v'/F_m'), D) Photochemical quenching (qL) and E) Non-photochemical quenching (NPQ) of six tree species submitted to three different light environments: full sunlight (open symbols); moderate shade (gray symbols); deep shade (black symbols). Values are mean ± standard error (n = 4).

In contrast to F_v'/F_m', photochemical quenching (qL) was higher for all species in FS, with emphasis on *T. serratifolia* and *B. grossularioides*, which were 9.4 and 5.1 times higher in qL than the plants in DS (Figure 2 D1-D3). With a higher necessity for energy dissipation in FS, NPQ followed the same behavior as qL, with the same species being 2.1 and 1.8 times higher in NPQ than that observed in for plants in DS. Both were followed by O*. pyramidale* which was 1.2 times higher in FS than those in MS (Figure 2 E1-E3).

The relative contributions of the ϕPSII were greater in the pioneers than in the other species in FS. In the other environments (MS and DS) the opposite was observed (Figure 3 A1-F-1). *C. guianensis* was the species that exhibited lower ϕPSII in all environments (Figure 3 B1-B3). However, the ϕNPQ did not differ between the species in FS and DS, but in MS *H. courbaril* was 11.8% lower than *B. grossularioides* and *C. guianensis*, which showed a higher value of ϕNPQ. Among the treatments, only *T. serratifolia* had higher ϕNPQ in FS (+12.2%) than DS.

Under normal conditions (21% O_2), the PPDF in which ϕNPQ exceeds ϕPSII (ϕPSII=ϕNPQ) was higher in FS plants than in DS plants, except for *C. guianensis* and *H. brasiliensis*, which did not differ between environments. In DS the species did not differ in this parameter, but in other environments *C. guianensis* exhibited the lowest ϕPSII=ϕNPQ values. In FS, the highest ϕPSII=ϕNPQ observed value was for *B. grossularioides* (about 564 μmol m^{-2} s^{-1}) and in MS for *H. courbaril* (about 517 μmol m^{-2} s^{-1}) (Figure 3). In general, under conditions in which photorespiration is theoretically suppressed (1% O_2), ϕNPQ exceeds ϕPSII at lower irradiances in FS, denoting the importance of this process in photochemical quenching. The extent to which the irradiance decreases between environments, ϕPSII increases, decreasing the participation of ϕNPQ (Figure 3).

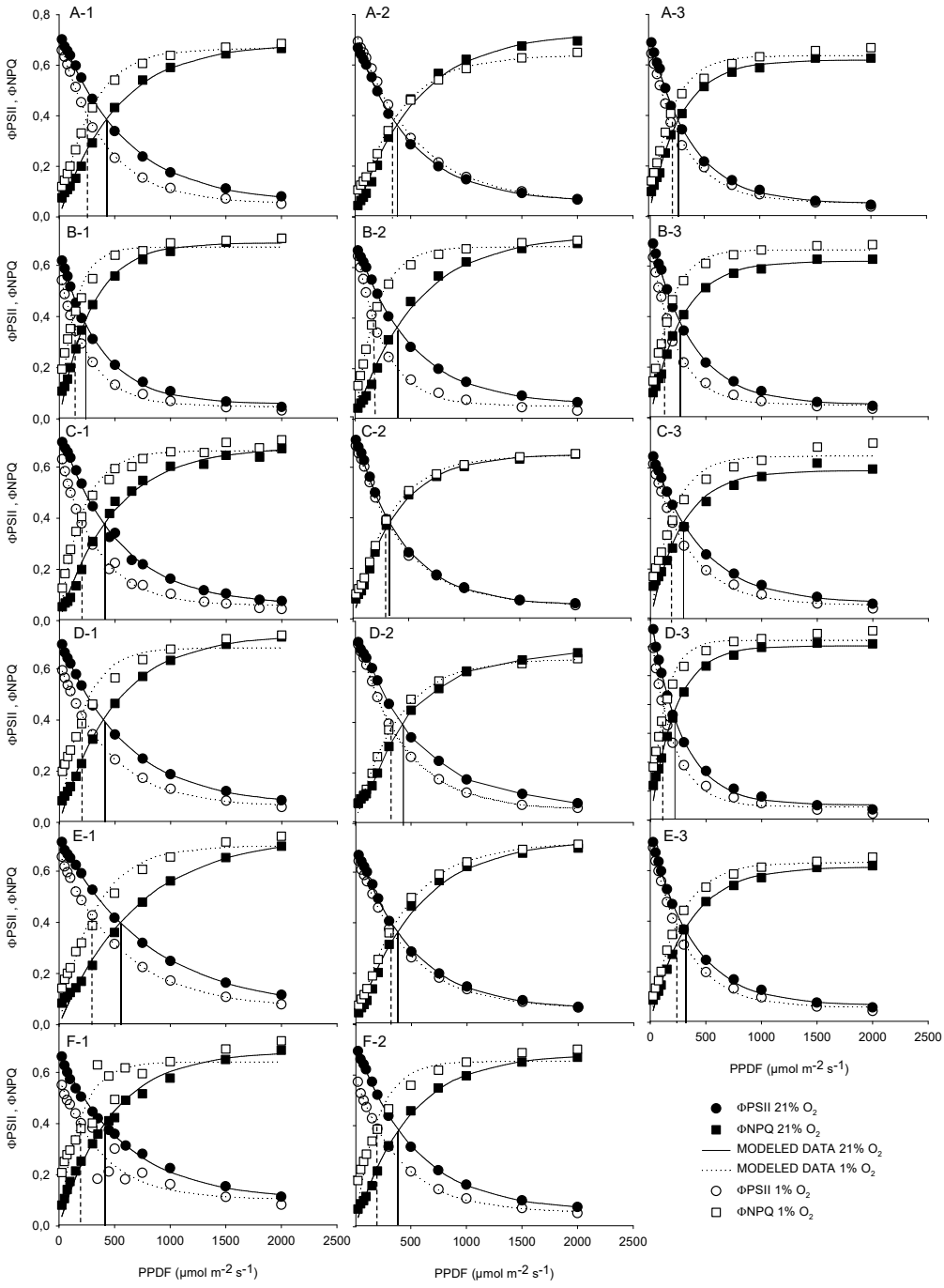

Figure 3: Photochemical and non-photochemical yields of absorbed energy with photosynthetic photon density flux (PPDF) [ΦPSII = m + aexp(–bPPF); ΦNPQ = m(1 – exp(–bPPF));] in seedlings: A) *Hymenea courbaril*, B) *Carapa guianensis*, C) *Hevea brasiliensis*, D) *Tabebuia serratifolia*, E) *Bellucia grossularioides* and F) *Ochroma pyramidale* subjected to three light environments: full sunlight (1); moderate shade (2); deep shade (3) and two O_2 levels. Vertical lines indicate PPF at which ΦPSII = ΦNPQ. Values are mean (n = 4).

3.3 *Enzymatic activities, leaf phenolic compounds and lipid peroxidation*

The major activities recorded for CAT were in *H. brasiliensis* in FS and MS. Only *C. guianensis* did not differ between treatments. The general trend of CAT activity suggests that higher light conditions induce higher activity of this enzyme, especially in non-pioneer species (Figure 4A). APX, on the contrary, displayed higher activity for *H. courbaril* and *C. guianensis* in DS than in FS (+ 99% and + 84.9%, respectively). Pioneer species exhibited less expressive results in all environments for this enzyme (Figure 4B).

The late successionals *H. courbaril* and *C. guianensis* also showed higher POX activity in DS than when in FS, as well as the pioneer *B. grossularioides* (+97.4%, +60.4% and +50.8%, respectively). Among the intermediates, *H. brasiliensis* did not differ between treatments and *T. serratifolia* showed lower activity in DS (-31.1%). For the pioneer *O. pyramidale,* the activity in MS was 56.8% higher than in FS (Figure 4C).

As POX, the SOD activity in DS was also higher in *H. courbaril*, *C. guianensis* and *B. grossularioides* than FS (5.8, 2.9 and 2.8 times greater). *H. brasiliensis* had higher SOD activity in MS 1.5 times higher in FS and 18 times higher in DS, whereas *T. serratifolia* in FS showed activity 2.4 and 1.4 higher than when in MS and DS, respectively. *O. pyramidale* did not differ between treatments (Figure 4D).

Phenolic compounds also have an antioxidant effect and, in general, the non-pioneer species exhibited higher total phenol content in FS than pioneer, especially the late sucessionals *H. courbaril* and *C. guianensis*; about 1.8 times higher than the others. For the pioneers, the highest values observed were in MS. *B. grossularioides* exhibited higher phenol content in DS and the lowest content in this environment were found in *H. brasiliensis* and *T. serratifolia* (-72.6%) (Figure 4E).

The test for lipid oxidation was evaluated by thiobarbituric acid reactive substances (TBARS) and the results were lower in DS than FS, with the exception of *C. guianensis*, which did not differ between these environments. *O. pyramidale* exhibited the lowest content of TBARS in FS and the highest values were observed for *B. grossularioides* and *H. courbaril*. In DS and MS, *B. grossularioides* had lower TBARS while *C. guianensis* in MS had higher TBARS than the others in this environment (Figure 4F).

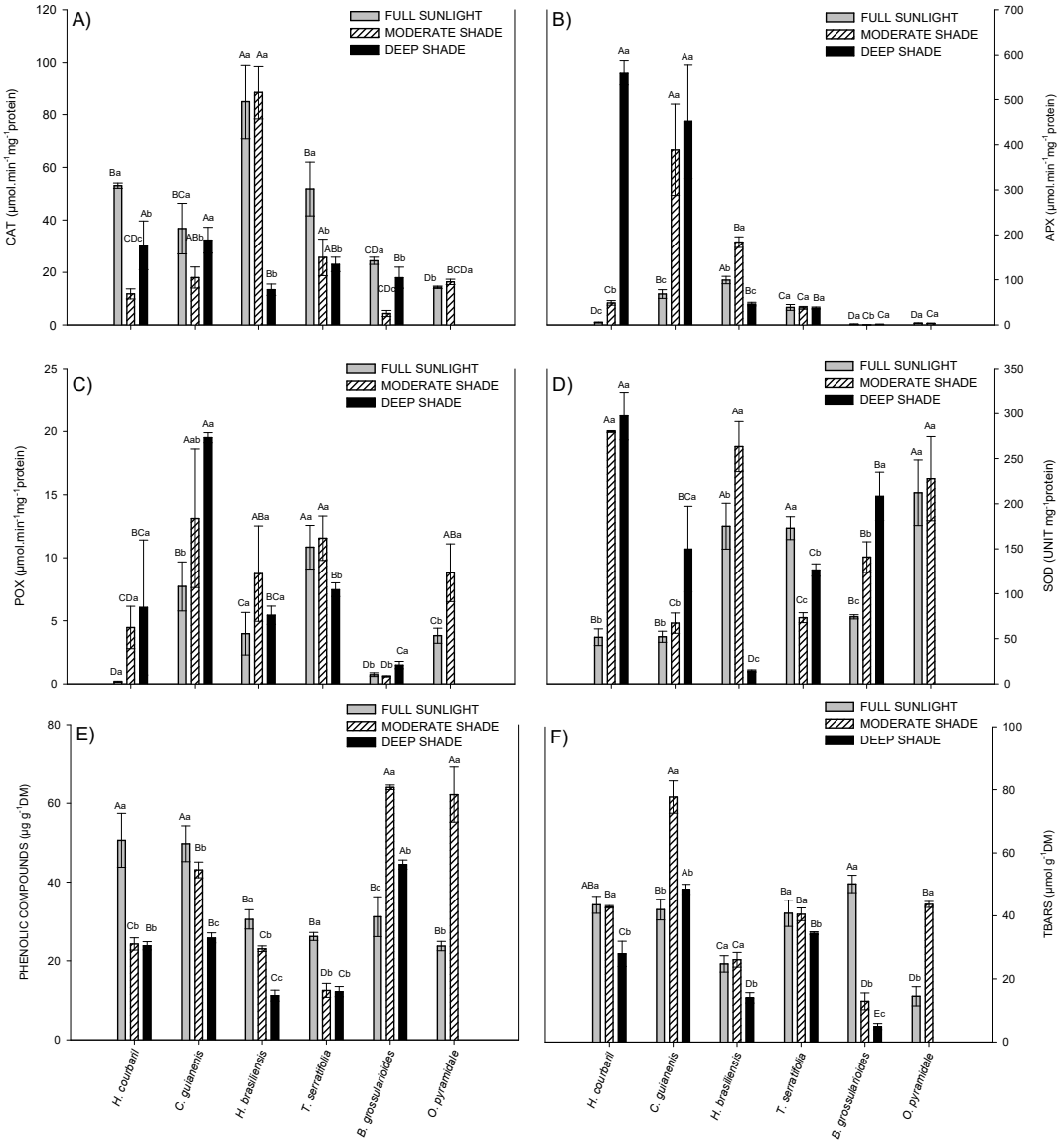

Figure 4: Antioxidant Activity of Enzymes A) Catalase (CAT), B) Ascorbate Peroxidase (APX), C) Phenolic Peroxidase (POX) and D) Superoxide Dismutase (SOD), E) Leaf phenolic compounds and F) Lipid Peroxidation Intensity (TBARS) of six tree species submitted to three different light environments. Same capital letters for different species in same environment and lower case for same species in different environment are equal by Tukey test ($p < 0.05$). Vertical bars indicate the standard error (n=4).

3.4 *Relationships between fluorescence parameters, antioxidant activity and leaf nutrient contents*

In general, the photochemical parameters in FS were more related to the content of P than in other environments. It is important to highlight the photoprotective role of CAR (r = -0.73) and the SOD (r = 0.74) activity acting together to reduce oxidative damage (TBARS). The strong relationship between N and enzymatic activity and especially of Fe with SOD activity (r = 0.64) is also observed (Figure 5A).

Interspecific responses to light intensity in each environment were evaluated using PCA. In FS, the PCA explained 45.44% of the variation of the data. Axis 1 of the PCA explained 23.79% of the data and (0.6 <r <0.9) (strong and positive). Strong and negative correlations were observed in this axis with ϕPSII, ETR and the leaf contents of Mg and P. Axis 2 explained 20.75% of data variation and the very strong positive correlations (r>0.9) were with SOD activity and strong correlations with phenolic contents, Chl*a*, Chl*b*, CAR and Chl_{tot} on mass basis. Strong negative correlations were between axis 2 and N foliar concentration (Figure 5B).

In contrast to FS, in MS there was lower participation of P in photochemical processes, especially in ϕPSII and ETR. The activity of SOD and the phenolic compounds performed discretely in the decrease of the damage (Figure 6A).

The PCA for MS explained 51.08% of the variation of the data and in axis 1 is contained 30.48% of the power of explanation. With this axis the contents of pigments, F_v'/ F_m', ϕPSII, ETR, N and Mn contents and ϕPSII=ϕNPQ were strongly correlated. In this axis, the strong and negative correlations were with the content of phenols and ϕNPQ. Axis 2 explained 20.60% of the data and was strongly related to APX and POX activity, Chl*a* : Chl*b* and Ca content. Negative correlation was observed with qL (Figure 6B).

In DS, there was a negative correlation between N content and enzymatic activity, as opposed to environments with higher irradiance, evidencing the investment of N and P foliar in light capture. Therefore, the antioxidative system had lower participation in protection against oxidative damage (TBARS), that was reduced with increases in ϕPSII, ETR and also by the maintenance of higher PSII yield at higher irradiance (ϕPSII = ϕNPQ) (Figure 7A).

The PCA for DS explained 53.30% of the variation of the data. Axis 1 contains 32.58% of the explanation and was strongly and positively related to the activity of CAT, TBARS, Chl*a*: Chl*b* and Chl_{tot}: CAR. Negative correlations occurred between axis 1 and pigment content, ϕPSII, ETR, contents of P and Zn and ϕPSII = ϕNPQ. With axis 2, containing 20.72% of the data variation, the positive correlations were with F_v'/ F_m', ϕNO and the contents of K and Fe. Negative correlations were between NPQ and ϕNPQ (Figure 7B).

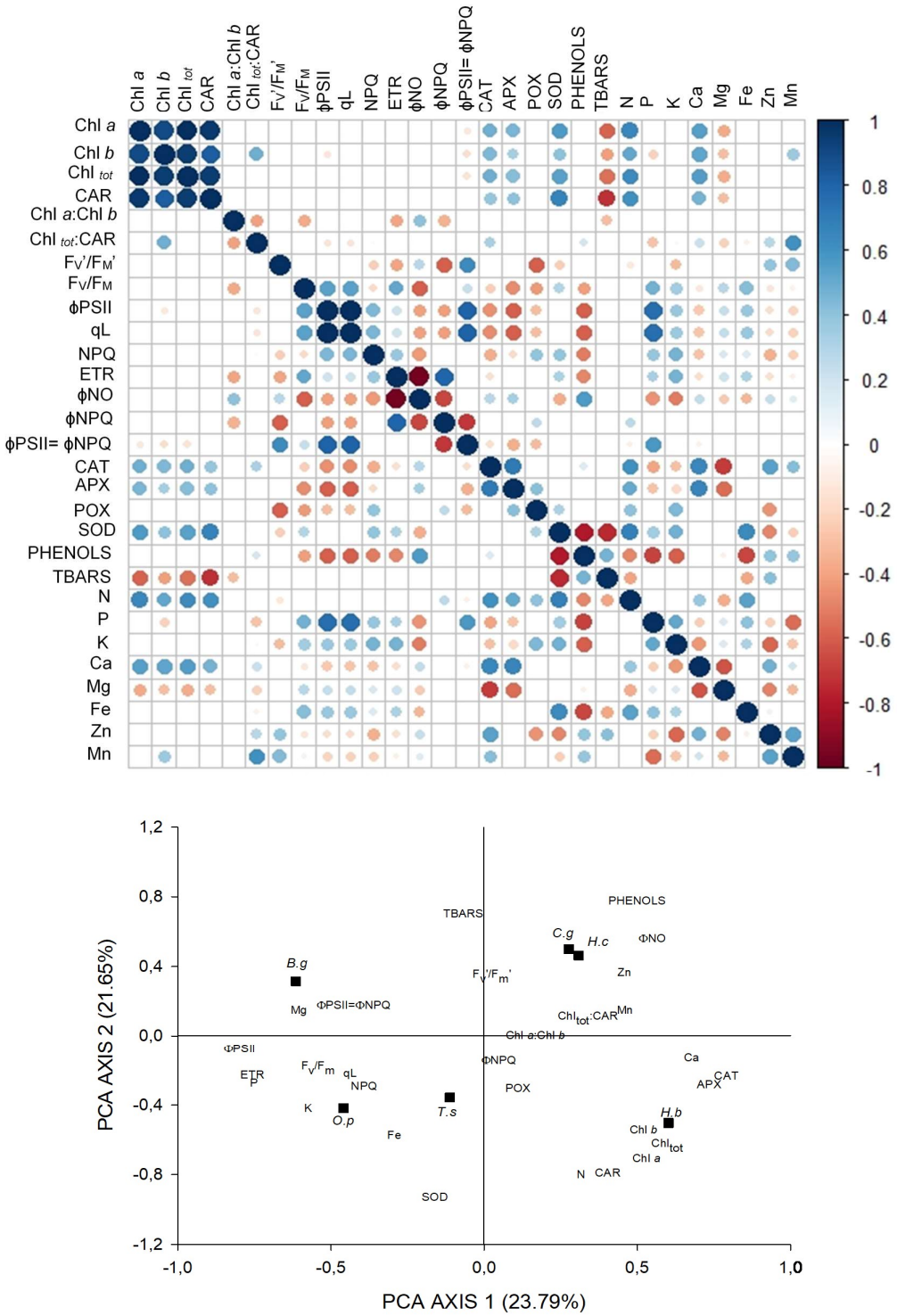

Figure 5: Correlations between pigments contents, fluorescence parameters, antioxidant enzymatic activity, phenolic compounds, lipid peroxidation and foliar concentration of nutrients in six tree species under full sunlight and principal components analysis of the 29 variables studied. Black squares represent the species: *Hymenea courbaril (H.c); Carapa guianensis (C.g); Hevea brasiliensis (H.b); Tabebuia serratifolia (T.s); Bellucia grossularioides (B.g) and Ochroma pyramidale (O.p).*

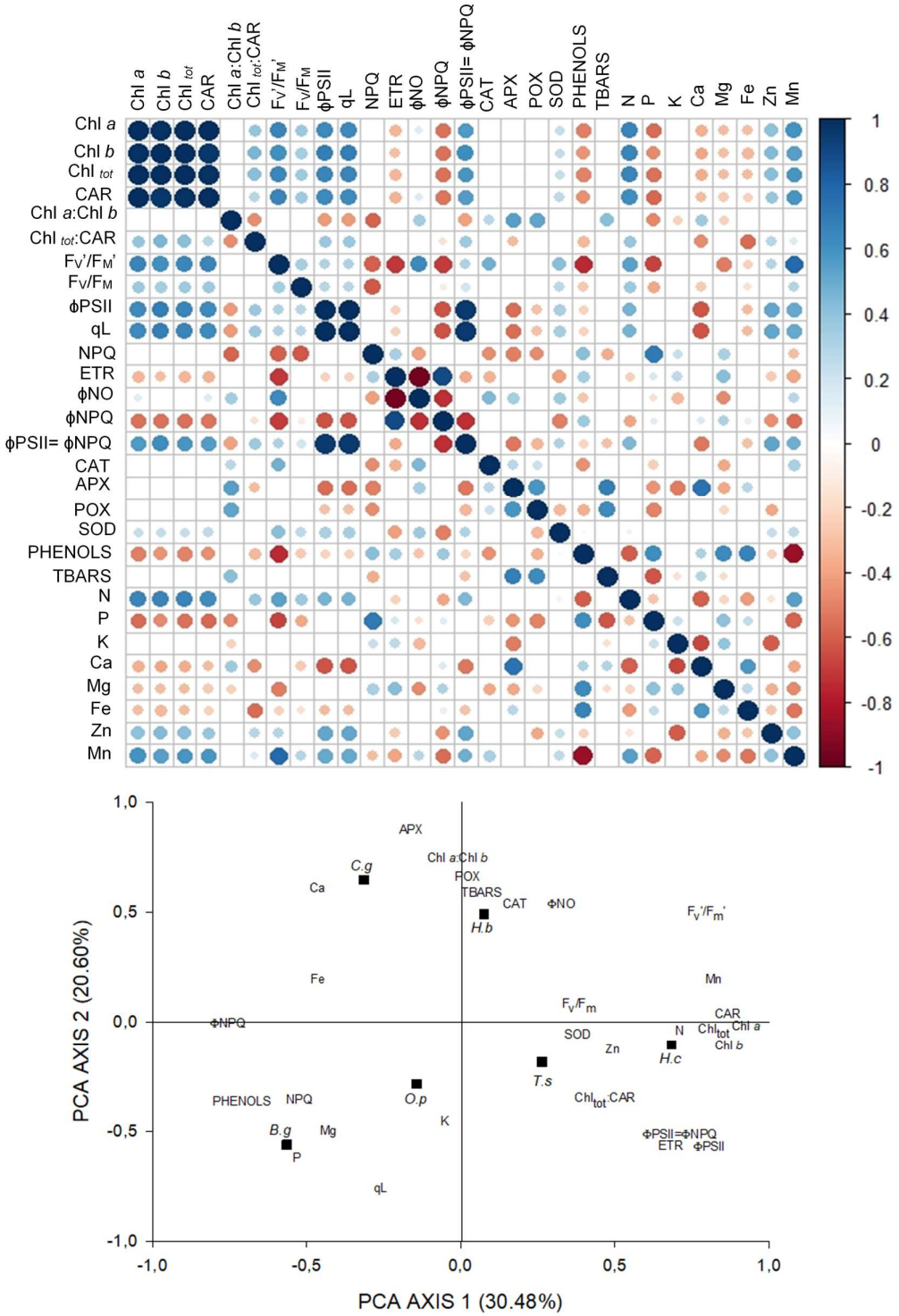

Figure 6: Correlations between pigments contents, fluorescence parameters, antioxidant enzymatic activity, phenolic compounds, lipid peroxidation and foliar concentration of nutrients in six tree species under moderate shade and principal components analysis of the 29 variables studied. Black squares represent the species: *Hymenea courbaril (H.c); Carapa guianensis (C.g); Hevea brasiliensis (H.b); Tabebuia serratifolia (T.s); Bellucia grossularioides (B.g) and Ochroma pyramidale (O.p)*.

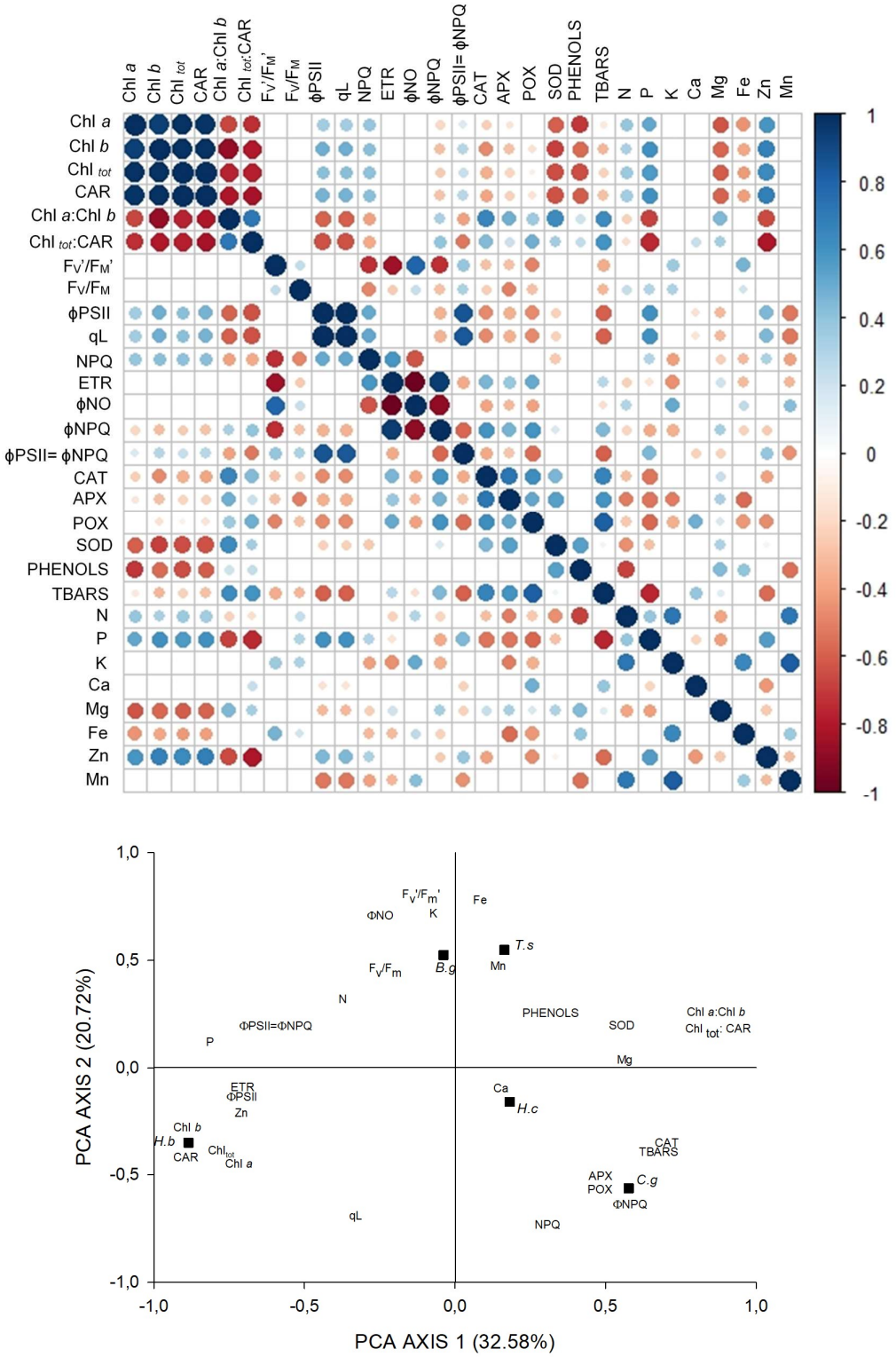

Figure 7: Correlations between pigments contents, fluorescence parameters, antioxidant enzymatic activity, phenolic compounds, lipid peroxidation and foliar concentration of nutrients in six tree species in deep shade and principal components analysis of the 29 variables studied. Black squares represent the species: *Hymenea courbaril (H.c); Carapa guianensis (C.g); Hevea brasiliensis (H.b); Tabebuia serratifolia (T.s); Bellucia grossularioides (B.g) and Ochroma pyramidale (O.p).*

4 DISCUSSION

4.1 Chloroplastidic pigment contents

Photosynthetic pigments are constantly degraded and synthesized in the presence of light, but under conditions of high irradiance the degradation occurs at a higher intensity than the synthesis, justifying the lower concentrations of pigments found in FS and this corroborates with the results of other studies (Magalhães et al., 2009; Favaretto et al., 2011; Gonçalves et al., 2012; Quevedo-Rojas et al., 2018). In addition, the reduction of pigment contents suggests a smaller light harvest complex, which may prevent the absorption of excess light energy and avoid oxidative damages. On the other hand, in DS, there was an increase in pigment content with the aim of increasing the light interception surface in order to compensate for the low availability of the resource in this environment.

The higher content of Chl *b* in DS favors the absorption of light at wavelengths higher than those of Chl *a*, which is more abundant in the diffuse radiation that reaches the understory. This change in the ratio between the two pigments makes the Chl *a*: Chl *b* ratio an important indicator of the plants adaptability to different light environments (Krause et al., 2001). As expected, Chl *a*: Chl *b* was higher in DS than in other environments.

The DS plants also exhibited a higher *Car* concentration, probably with the same objective of increasing Chl *b*, increasing the absorption of light at other wavelengths. Despite the higher content in DS, the Chl_{tot}:CAR ratio was higher in FS, demonstrating the higher proportion of carotenoids in relation to chlorophylls in this environment given its important photoprotective role. The increase of these pigments in the xanthophyll cycle is fundamental for the dissipation of excess energy under high irradiance conditions (Gonçalves et al., 2001).

The observed results corroborate the remarkable capacity of the studied plants to change the N fraction in light capture (P_I) observed in Chapter 2 (Figure 4E), demonstrating plasticity at least regarding the flexibility of the collector complex between the environments.

4.2 *Chlorophyll fluorescence parameters*

Among the species studied, only *C. guianensis* exhibited photoinhibition effects, evidenced by the lower F_v/F_m ratio (0.67), and this occurred in FS where the values displayed were below 0.75. Values close to 0.80 indicate maximum efficiency in the use of energy in the photochemical process while values below 0.75 indicate a stress situation in which there is a reduction of the photosynthetic potential of the plant (Maxwell and Johnson, 2000). Other studies have reported a reduction of the F_v/F_m ratio in late successional species, including *C. guianensis*, when submitted to high irradiance (Morais et al., 2007; Gonçalves et al., 2010; Azevedo and Marenco, 2012).

The highest ETR observed for pioneer species in FS is compatible with the high photosynthetic rates exhibited (see Chapter 2), and these values can be associated with the high energy availability in the environment since the lowest ETR were observed in saplings subjected to DS. In plants under stress, when the electron flux in the photochemical stage is intense, alternative routes to carboxylation can be activated to dissipate excess energy preventing photochemical damage, and photorespiration is one of the main ways of consuming excess energy (Voss et al., 2013). The highest total ETR in FS was accompanied by a higher flow of electrons destined for photorespiration in this environment, notably for *C. guianensis* and *T. serratifolia*, which exhibited 10% and 7% higher FS photorespiratory costs when compared to plants in DS.

The efficiency of photosystem II in light (F_v'/F_m') and the photochemical extinction coefficient (qL), as ETR, were strongly influenced by the light environment. These parameters, which represent the portion of the excitation energy captured by the open FSII reaction centers and the proportion of electrons used in the photochemical phase, indicate that the plants are able to exhibit the use of the ambient radiation, although damage occurred to *C. guianensis*. It can be seen that although FS plants have a lower portion of open PSII they exhibit the higher qL under saturating light, resulting in greater energy available for the Calvin cycle. This increase in qL may be a consequence of the higher rates of electron transport around the photosystems in FS.

Only *T. serratifolia* and *B. grossularioides* exhibited differences in NPQ between FS and DS under saturating light. The induction kinetics of NPQ triggered by saturating light generally have a typical time dependence: they increase after illumination due to the initiation of electron transport and formation of NADPH preceding the activation of ATP synthase and decrease again when the Calvin cycle is activated (Murchie and Lawson, 2013; Kalaji et al., 2014). In this sense, it is possible to notice activation of the slower Calvin cycle in FS plants, corroborating the data found in Chapter 2 (Figure 4E).

The ϕPSII is intrinsically associated with the non-cyclic electron transport rates, so that the lower ϕPSII observed for *C. guianensis* in FS and *T. serratifolia* in DS affected the ETR and consequent photosynthesis in these environments. This parameter measures the proportion of light absorbed by the PSII associated chlorophylls that are effectively used in photochemical processes, and, as well as the F_v/F_m, can be used as an indicator of plant performance under different types of stress (Guidi and Calatayud, 2014; Schimpl et al., 2018).

The relative contributions of the photochemical (ϕPSII) and non-photochemical (ϕNPQ) processes for absorbed energy processing were quite divergent environments. For *B. grossularioides* in FS and *H. courbaril* and *T. serratifolia* in MS, the thermal dissipation was required in irradiance superior to the other species, corroborating with the best photochemical and photosynthetic performance of these species in the respective environments. Higher ϕPSII contributions compared to ϕNPQ indicate higher photochemical dissipation capacity without dependence on thermal dissipation, which is interesting for the studied species, since NPQ has little difference among environments. Thus, efficient performances of photochemical extinction

mechanisms are fundamental to dissipate excess energy and prevent damage. In this sense, it is emphasized that under low O_2 conditions, in which photorespiration is suppressed, ϕNPQ surpassed ϕPSII in lower irradiance in all species and environments, independent of successional groups, evidencing that the absence of this process overloads the photochemical processes and renders the plants more susceptible to oxidative damage, which reinforces, once again, the importance of this process in the dissipation of excess energy.

4.3 *Enzymatic activities, leaf phenolic compounds and lipid peroxidation*

Physiological stresses can lead to disturbances in plant metabolism which increase the production of reactive oxygen species (ROS) and may cause oxidative damage (Alexieva et al., 2001). Plant tolerance to stress factors is associated with its antioxidant capacity, and increasing levels of antioxidant constituents can prevent stress damage. Thus, an efficient antioxidant system is fundamental for the protection of the photosynthetic apparatus under conditions of stress generated by high irradiance (Noctor et al., 2015).

In a study comparing the antioxidant system of tree species in different light levels, Favaretto et al. (2011) concludes that the best acclimation of pioneer species to high irradiance environments is in part due to higher antioxidant enzymatic activity of this group in comparison to non-pioneer species. Contrasting with these results, no clear pattern was observed among successional groups, nor greater antioxidant enzymatic activity of two pioneer species studied.

The enzymes CAT, APX and POX belong to different classes of H_2O_2 dissipation enzymes, however, CAT is indicated as responsible for more efficient elimination and more of this compound (Jaleel et al., 2009; Barbosa et al., 2014). In general, higher CAT activities were found in higher irradiance environments, except for *C. guianensis*, which did not differ in FS and DS (36 and 32 μmol min^{-1} mg^{-1} of protein, respectively). The opposite was observed for APX, where *H. courbaril* and *C. guianensis* had higher activity in DS (560 and 451 μmol min^{-1} mg^{-1} of protein, respectively). The enzyme CAT has a higher occurrence in peroxisomes due to the photorespiratory process and APX occurs mainly in chloroplasts (Yanik and Donaldson, 2005). Due to the higher H_2O_2 formation as a consequence of photorespiration, higher CAT activity may be expected in environments with higher photorespiratory rates, in this case in FS, and higher APX activity under low irradiance (DS), where H_2O_2 is formed in a smaller amount (Sousa et al., 2019).

This modification of the balance of enzyme activity in stress situations can lead to compensatory mechanisms, with suppression of activity of one enzyme inducing the synthesis of another with the same purpose (Apel and Hirt, 2004). Although the role of APX in the elimination of H_2O_2 is recognized, CAT is considered more effective, which makes their greater activity in FS necessary (Sousa et al., 2019). In spite of the lower activity of pioneer species, *B. grossularioides* and *O. pyramidale* had higher proportional activity of CAT in relation to APX in FS (17 and 4 times, respectively), demonstrating a high capacity to modulate the performance of these enzymes in

function of the irradiance environment, contributing to the integrity (structural and functional) of the photosynthetic machinery.

The POX, as well as CAT and APX, play an important role in the antioxidative system detoxifying H_2O_2 dismutted by SOD, however, POX had less activity than two previous ones. Both APX and POX have several isoforms and are present in several compartments (Jaleel et al., 2009). While CAT catalyzes the direct reduction of H_2O_2 to H_2O and O_2, APX eliminates H_2O_2 at expense of ascorbate and POXs, thus reducing phenols. In this regard, it is believed that the main importance of POX is the oxidation of several substrates in the presence of H_2O_2 that could become reactive (Bela et al., 2018). Similar to APX, the enzyme POX had less activity among the pioneers.

In regards to SOD, while Favaretto et al. (2011) and Hansen et al. (2002) found higher SOD activity in plants of pioneer species and full sunlight acclimated leaves, in this study the results did not present a very clear pattern. Both late successional species and the pioneer *B. grossularioides* exhibited lower SOD activity in FS and higher activity in DS, the intermediates presented lower activity in DS and the pioneer *O. pyramidale* did not differ between FS and MS.

SOD is considered the first line of defense in the fight against ROS, transmuting $O_2\bullet^-$ to form H_2O_2, since it is the complete detoxification of the free radicals complemented by the CAT, APX and POX enzymes. Although it is ubiquitous in aerobic organisms and subcellular compartments prone to oxidative stress, SOD relative abundance varies greatly among plants (Gill and Tuteja, 2010; Barbosa et al., 2014; Wu et al., 2015).

Phenolic compounds are also part of the antioxidant system (Jaleel et al., 2009), especially absorbing UV radiation and reducing damages caused by high irradiance. However, in this study they had no significant participation in the prevention of cellular damage. The lipid peroxidation was estimated by the measurement of the formation of reactive substances to thiobarbituric acid (TBARS) and it was observed that the highest content of TBARS was found in plants in higher irradiance environments. The formation of TBARS in these plants is comparable to other species studied under stress conditions (Gill and Tuteja, 2010; Noctor et al., 2016; Lima et al., 2018). It is important to note that, despite the indications for lipid peroxidation, the functional stability of PSII was maintained for all plants except for *C. guianensis* in FS, as verified by the F_v/F_m ratio, so the TBARS content should not be taken solely for assessment of the effectiveness of antioxidative system.

4.4 *Relationships between fluorescence parameters, antioxidant activity and leaf nutrient contents*

In general, in all three environments, the N content was positively related to pigment contents. The positive correlation of N with the enzymatic activities in FS demonstrates the importance of the antioxidant system for the control of ROS and cellular damage, especially by

the activity of SOD and by the action of carotenoids. The activity of SOD in FS was positively related to the Fe content; a component of the prosthetic group in the chloroplasts (Noctor et al., 2016).

The positive relations between P content and the photochemical processes (F_v/F_m, ETR and PSII) related to efficiency in energy capture and transfer were notably higher in FS due to the higher occurrence of these events in the environment with higher irradiance. The proper functioning of light energy conversion is an indication of the adequate supply of this nutrient in the leaves (Kalaji et al., 2016). It is important to emphasize the participation of the micronutrients Mn and Fe in the increase of F_v'/F_m' and F_v/F_m, respectively. The first has fundamental participation in the complex evolution of oxygen and the second, although acting more clearly in the electron transport chain via its metallic nature, can help to relieve the excess energy and participate effectively in the redox systems or redox signaling in the plant cells.

Positioning on the negative side of axis 1 of PCA in FS demonstrates the best photochemical performance of the pioneer species in this environment, evidenced by the higher ϕPSII, ETR and qL. The lower antioxidant enzyme activity in *B. grossularioides* appears to have been compensated for by phenolic compounds, but was insufficient to reduce oxidative damage (TBARS). The lower damage in *O. pyramidale* (14.5 μmol g^{-1} DM) seems to be a consequence of the greater activity of SOD (212.2 Unit mg^{-1} of protein).

With an irradiance decrease in MS and DS, the effect of SOD activity in reducing TBARS also decreases. This result suggests a convergence (SOD activity and lipid peroxidation) at lower energization states. In MS, the participation of P in photochemical processes decreased in relation to FS, contributing only to the increase of qL. However, the negative P-TBARS relationships indicate that this element contributed to the reduction of cellular damage, probably increasing the efficiency of reactions that require energy transfer mediated by ATP. The positive effects of Mn on fluorescence parameters were repeated in MS, with emphasis on the increase of ϕPSII=ϕNPQ, probably because this conferred greater stability to the functioning of PSII.

The positioning of *H. brasiliensis* and *B. grossularioides* in in PCA of MS suggests that the lower oxidative damages observed in these species are due to the higher antioxidant enzymatic activity in the first (CAT) and to the high qL and NPQ for the second.

In DS ϕPSII = ϕNPQ this parameter was also important, where, together with ETR and ϕPSII, the negative correlations with TBARS show that efficient mechanisms of photochemical extinction in plants under intense shading may be more decisive in the prevention of oxidative damage than a strong antioxidant system, since this would require an investment of part of the nitrogen allocated to capture and transfer energy (Rodríguez-Calcerrada et al., 2008).

PCA in DS allows us to infer that the higher ϕPSII in *B. grossularioides* and *H. brasiliensis* can be attributed, at least partially, to the higher pigment contents and lower dissipations regulated and not regulated by PSII (ϕNPQ and ϕNO, respectively), as well as to the increase in P contents.

5. CONCLUSION

The antioxidant system presented few significant differences between groups, but it was observed that the late-successional species exhibited higher phenolic content and lower SOD activity under full sunlight, diverging from the intermediate and pioneer ones.

Under high irradiance, the enzymatic activity of SOD was determinant for the control of TBARS. The low use of N for constitution of large light harvest complex in FS allowed the constitution of a robust antioxidant enzymatic system while in the plants under shade the need to increase the light harvest complex reduced the investments of N in this sense. Thus, the most efficient way of avoiding oxidative damage to plants under intense shade was the increase of photochemical quenchings and the use of carotenoids for thermal dissipation via the xanthophyll cycle. In this respect, we highlight the positive effect of P on the increase of PSII yield in all environments, especially those with higher irradiance.

Finally, the pioneer species presented better photochemical performance under high irradiance compared to non-pioneer species. The late successional *C. guianensis* was the only one that showed signs of photoinhibition and the other species, despite the signs of lipid peroxidation in FS, exhibited adequate PSII yield and showed themselves to be able to withstand the high irradiance. On the other hand, the death of *O. pyramidale* and the increased SOD activity in DS recorded for *B. grossularioides* suggest that the stress due to low light availability for pioneer plants may be more difficult to overcome than the high irradiance for late-successional species.

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GENERAL CONCLUSIONS

The ability of saplings to accumulate biomass did not follow a pattern between successional groups, and the relationship between higher photosynthetic rates and higher biomass accumulation that occurred in full sun does not apply to the shaded environment, where apparently different strategies for NSC accumulation, turnover and allocation are more decisive than high photosynthetic rates.

The highest photosynthetic rates occurred in the higher irradiance environment and the reduction of photosynthesis is governed by diffusive limitations, with little or no influence of metabolic retroinhibition associated to the accumulation of final products, regardless of the successional group or the species' growth strategy.

For acclimation to light environments, the partition of leaf nitrogen between photosynthetic and structural compounds was more determinant for photosynthesis than its own content and the capacity to modulate the N allocation is fundamental for the increase or decrease of photosynthesis due to changes in irradiance.

The late successional *C. guianensis* was the only species to exhibit signs of photoinhibition under full sunlight. The other species, despite the signs of lipid peroxidation, showed adequate PSII yield and were able to withstand the high irradiance. The control of oxidative damage in full sunlight was mainly due to the greater activity of SOD (the efficient ROS scavenging could reduce the damage to membrane lipids), while in shaded environments the improvement of the processes involving transfer and dissipation of light energy were shown to be more efficient in the control of photoinhibition.

The results show that the photosynthetic plasticity of acclimation to irradiance is not related to the successional group, but is dependent on the individual capacity of the species to modulate functional characteristics of the leaves, mainly those related to the acquisition (individual leaf area and leaf gain) and resource allocation (leaf N partition) depending on the availability of light.

Finally, as a silvicultural recommendation, only *C. guianensis* indicated a lower tolerance to high irradiance, and should be cultivated in a moderately shaded environment. All other studied species are suitable for planting in full sunlight.

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APENDIX

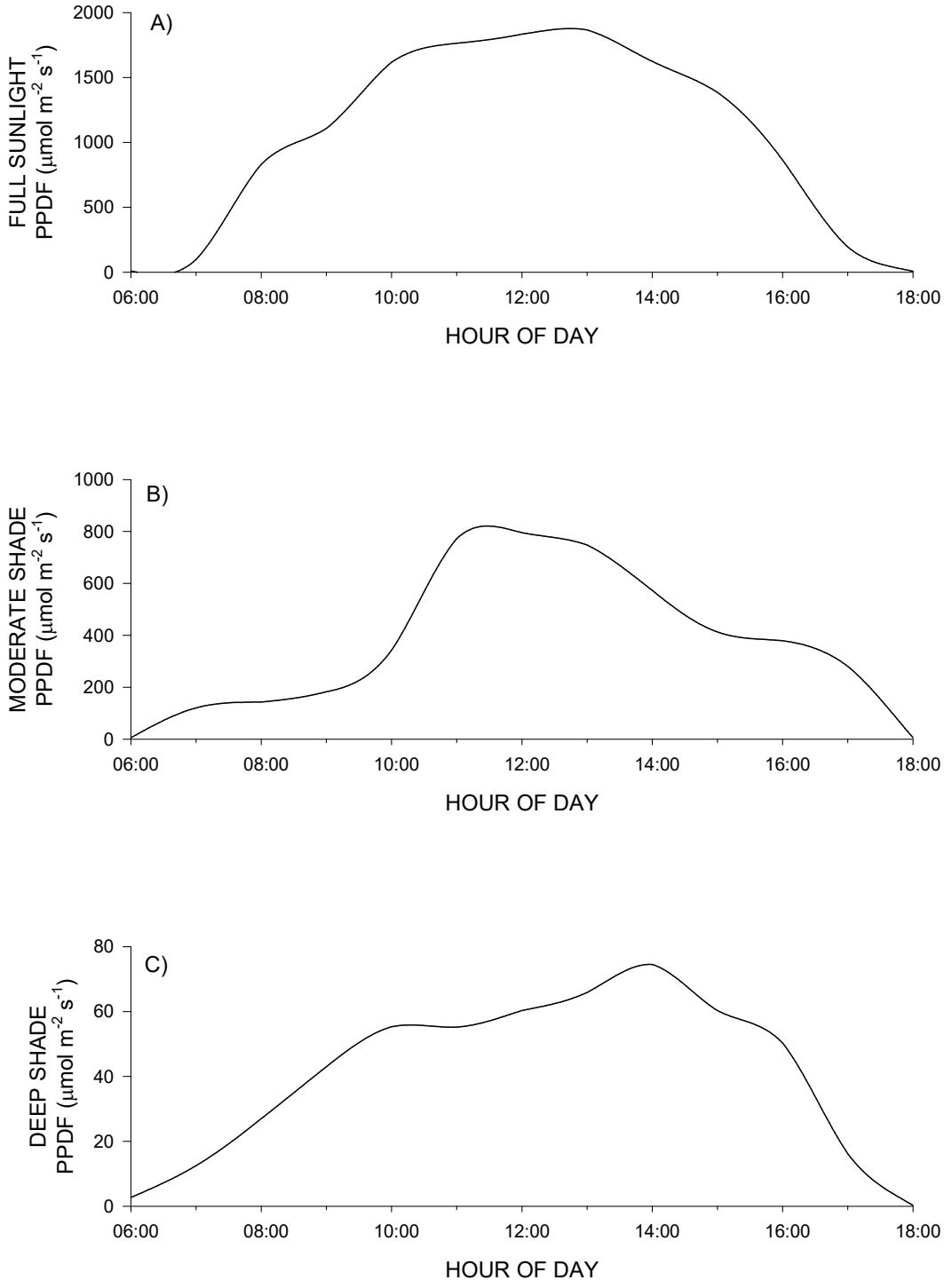

Figure 1: Distribution of photosynthetic photon flux density (PPDF) along the day in three different light environments: A) FS - full sunlight, B) MS – moderate shade and C) DS- deep shade.

Table 1. Relative growth rate in high (RGR-h), diameter (RGR-d), leaf area (RGR-la), leaf gain index (LGI), net assimilatory rate (NAR)e specific leaf area (SLA) in saplings of six tree species submitted to different light environments.

ENVIRONMENT	SPECIE	RGR-h (cm cm^{-1} dia^{-1})	RGR-d (mm mm^{-1} dia^{-1})	RGR-la (cm^2 cm^{-2} dia^{-1})	IGF (%)	NAR (g cm^{-2} dia^{-1})	SLA (cm^2 g^{-1})
FULL SUNLIGHT	*H. courbaril*	0.00132 ±0.00022 Ba	0.00306 ±0.00035 Ba	0.00471 ±0.00200 Aa	80.75± 23.43 Aa	0.00055 ±0.00011 Ba	132.42 ±12.98Cb
	C. guianensis	0.00066 ±0.00014 Ca	0.00291 ±0.00028 Ba	0.00129 ±0.00070 Ba	22.30±1.53 Ca	0.00033 ±0.00003 Ba	120.00 ±15.22Cc
	H. brasiliensis	0.00059 ±0.00014 Ca	0.00318 ±0.00023 Ba	-0.00180 ±0.00034 Ca	33.06±10.17BCa	0.00047 ±0.00009 Ba	196.07 ±13.16Bb
	T. serratifolia	0.00252 ±0.00037 Aa	0.00333 ±0.00082 Ba	0.00123 ±0.00036 Ba	30.19 ±8.19BCa	0.00034 ±0.00006 Ba	182.11 ±4.03Bb
	B. grossularioides	0.00089 ±0.00028BCa	0.00352 ±0.00040ABa	0.00134 ±0.00017 Ba	57.19±14.99ABa	0.00045 ±0.00013 Ba	109.75 ±10.16Cb
	O. pyramidale	0.0006 ±0.00010Cb	0.00440 ±0.00035Ab	-0.00175 ±0.00035Cb	69.81 ±12.94 Aa	0.00107 ±0.00022 Aa	237.20 ±11.09Ab
MODERATE SHADE	*H. courbaril*	0.00179 ±0.00056ABa	0.00288 ±0.00070 Aa	0.00300 ±0.00130 Aa	37.69 ±8.32ABb	0.00047 ±0.00005 Aa	142.87 ±27.67Bb
	C. guianensis	0.00086 ±0.00037 Ca	0.00291 ±0.00027 Aa	0.00285 ±0.00136 Aa	20.67 ±3.52 Ba	0.00019 ±0.00003Cb	155.95 ±17.92Bb
	H. brasiliensis	0.00107 ±0.00013 Ba	0.00260 ±0.00045 Aa	-0.00176 ±0.00037 Ca	38.89 ±16.36ABa	0.00026 ±0.00003BCb	214.68 ±74.97Bb
	T. serratifolia	0.00193 ±0.00031 Aa	0.00248 ±0.00071 Aa	0.00051 ±0.00016 Ba	34.43 ±4.34ABa	0.00021 ±0.00005BCb	228.85 ±37.98Bb
	B. grossularioides	0.00102 ±0.00038 Ba	0.00255 ±0.00060Ab	0.00165 ±0.00031ABa	40.84 ±9.31ABa	0.00022 ±0.00006BCb	143.29 ±25.00Bb
	O. pyramidale	0.00117 ±0.00013ABa	0.00244 ±0.00081Ab	0.00266 ±0.00076 Aa	50.56 ±12.37 Aa	0.00031 ±0.00010Bb	327.80 ±20.06 Aa
DEEP SHADE	*H. courbaril*	0.00053 ±0.00021Bb	0.00098 ±0.00015ABb	0.00278 ±0.00098 Aa	19.25 ±6.75Ab	0.00007 ±0.00002Ab	402.61 ±50.77 Ba
	C. guianensis	0.00105 ±0.00043ABa	0.00133 ±0.00019Ab	0.00140 ±0.00019 Aa	5.40 ±0.90Ab	0.00002 ±0.00002ABc	297.85 ±29.28 Ca
	H. brasiliensis	0.00068 ±0.00043ABa	0.00137 ±0.00038Ab	-0.00180 ±0.00004 Ba	8.33 ±16.67Ab	-0.00001 ±0.00002BCc	419.83 ±62.10 Ba
	T. serratifolia	0.00124 ±0.00030Ab	0.00065 ±0.00023Bb	-0.00210 ±0.00128Bb	27.50 ±10.95 Aa	-0.00006 ±0.00002Cc	520.60 ±31.15 Aa
	B. grossularioides	0.00067 ±0.00004ABa	0.00094 ±0.00032ABc	-0.00316 ±0.00167Bb	30.54 ±15.19Ab	-0.00006 ±0.00005Cc	294.18 ±41.14 Ca
	O. pyramidale						

Mean ± standard deviation (n=4) follow in lines for same capital letter to different species in same environment and lower case to same species in different environment are equal by Tukey test ($P \leq 0,05$).

Table 2. Leaf macro and micronutrient content of six tree species submitted to different light environments.

ENVIRONMENT	SPECIE	N ($gkg^{-1}DM$)	P ($gkg^{-1}DM$)	K ($gkg^{-1}DM$)	Ca ($gkg^{-1}DM$)	Mg ($gkg^{-1}DM$)	Fe ($mgkg^{-1}DM$)	Mn ($mgkg^{-1}DM$)	Zn ($mgkg^{-1}DM$)
FULL SUNLIGHT	*H. courbaril*	10.67 ± 0.40BCc	1.04 ± 0.08Cb	2.87 ± 0.23Cb	20.88 ± 6.14 Ba	2.83 ± 0.75ABa	75.25 ± 10.31Bb	145.98 ±39.47 Aa	41.10 ± 9.66Ab
	C. guianensis	9.18 ± 0.28BCb	1.22 ± 0.27 Ca	4.06 ± 1.62BCa	21.30 ± 3.03Bb	3.85 ± 1.14 Aa	49.00 ± 10.80ABb	15.33 ± 1.63CDb	26.43 ± 6.25BCa
	H. brasiliensis	17.01 ± 2.51Ab	1.77 ± 0.39BCa	4.14 ± 1.65BCb	40.45 ± 14.42 Aa	1.13 ± 0.29 Ba	90.00 ± 9.20Ab	47.21 ± 10.28BCa	38.11 ± 5.00ABb
	T. serratifolia	16.63 ± 0.64Ab	1.75 ± 0.08BCa	9.86 ± 0.70 Ac	16.82 ± 5.05 Ba	3.44 ± 1.06 Aa	97.00 ±10.30Ab	69.54 ± 8.31 b	20.51 ± 4.13 b
	B. grossularioides	8.87 ± 0.55Cb	2.72 ± 0.41Ab	6.79 ± 2.14ABb	14.84 ±2.26Bb	3.96 ± 0.48 Aa	82.00 ± 19.13Ab	8.67 ± 0.95Db	31.90 ± 6.58ABCb
	O. pyramidale	12.03 ± 1.73 Ba	2.26 ± 0.57ABb	7.04 ± 2.22ABb	12.70 ± 1.50 Ba	5.06 ± 1.72 Aa	94.25 ± 18.66 Aa	8.70 ± 3.35 Da	11.28 ± 2.92 Da
MODERATE SHADOW	*H. courbaril*	15.07 ± 0.39ABb	1.27 ± 0.38 Ba	3.73 ± 0.25Cb	17.10 ± 3.99CDab	2.94 ± 1.39ABa	81.75 ± 10.44CDb	56.35 ± 14.27Ab	76.85 ± 14.99 Aa
	C. guianensis	10.30 ±0.65Bb	1.30 ± 0.26 Ba	4.47 ± 2.30 Ba	32.05 ± 2.35Aab	3.25 ± 0.75ABa	105.75 ± 13.50 Aa	22.70 ± 11.10 Bab	35.33 ±4.10 Ba
	H. brasiliensis	15.51 ± 3.70ABb	2.37 ±1.15 Ba	6.41 ± 1.79Bb	24.55 ± 2.24 Bab	2.42 ± 1.83 Ba	98.00 ± 11.46ABa	43.78 ± 7.02 Aa	21.68 ± 12.19BCb
	T. serratifolia	19.62 ± 5.51Ab	2.14 ± 0.49 Ba	15.93 ± 2.85Ab	6.78 ± 2.29Eb	3.28 ±1.77ABa	61.50 ± 5.32 Da	51.30 ± 10.81Ab	26.73 ± 3.93BCab
	B. grossularioides	9.63 ± 1.40Bb	6.68 ± 2.39 Aa	8.68 ± 0.66Bb	22.53 ± 2.70BCa	5.03 ± 0.63ABa	94.00 ± 10.42BCb	10.20 ± 2.51Bb	39.83 ± 6.18 Bab
	O. pyramidale	14.86 ± 2.91ABa	3.40 ± 0.73 Ba	16.47 ± 3.26 Aa	14.93 ± 2.71 Da	5.63 ± 0.64 Aa	119.50 ± 8.50Ab	17.68 ± 4.30Bb	12.38 ± 1.73 Ca
DEEP SHADOW	*H. courbaril*	20.48 ± 3.65 Ba	1.79 ± 0.30BCa	12.46 ± 3.27BCa	11.08 ± 2.19 Bab	4.75 ± 0.99 Aa	127.75 ± 32.50 Ba	46.48 ± 12.22Bb	68.78 ± 9.56ABa
	C. guianensis	15.41 ± 1.74 Ba	1.15 ± 0.30 Ca	6.06 ± 2.45 Ca	28.83 ± 7.83Aab	5.14 ± 2.99 Aa	110.75 ± 19.00 Ba	35.30 ± 9.42 Ba	28.55 ± 5.33 Ca
	H. brasiliensis	26.85 ± 2.93 Aa	2.66 ± 0.36 Aa	11.90 ± 3.64BCa	20.64 ± 7.32ABb	2.83 ± 0.26 Aa	114.00 ± 13.69 Ba	39.33 ± 9.87 Ba	77.78 ± 16.32 Aa
	T. serratifolia	31.98 ± 2.10 Aa	1.73 ± 0.26BCa	27.35 ± 3.72 Aa	21.81 ± 2.62ABa	3.99 ±1.01 Aa	225.00 ± 27.19 Aa	122.15 ± 19.29 Aa	32.38 ± 6.01 Ca
	B. grossularioides	18.63 ± 2.54 Ba	2.21 ± 0.57ABb	15.60 ± 3.94 Ba	19.28 ± 4.34ABab	5.18 ± 0.86 Aa	246.00 ± 51.48 Aa	28.40 ± 2.81 Ba	48.50 ± 3.47BCa
	O. pyramidale								

Mean ± standard deviation (n=4) follow in lines for same capital letter to different species in same environment and lower case to same species in different environment are equal by Tukey test ($p< 0,05$

Table 3: Pearson correlation (r) among photosynthetic parameters in tree species submitted to full sunlight (FS).

VARIABLES	R_d	α	LCP	A_{max}	LSP	ETR	R_P	E	WUE	IWUE	ϕPSII	g_s	g_m	V_{cmax}	J_{max}	J_{max}/V_{cmax}	P_r	P_b	P_l	P_s	SLA	LMA	SL	ML	BL	EUCa	EUMg	EUK	EU N	EUP	EUFe	EUZn	EUMn	EI(%)
R_d	**1.0**																																
α	0.1	**1.0**																															
LCP	**0.7**	-0.1	**1.0**																														
A_{max}	0.1	0.3	0.2	**1.0**																													
LSP	0.0	-0.1	0.1	0.3	**1.0**																												
ETR	0.1	0.1	0.2	**0.9**	**0.4**	**1.0**																											
R_P	0.0	0.1	0.1	**0.8**	**0.5**	**0.8**	**1.0**																										
E	0.0	0.2	0.2	**0.9**	0.3	**0.9**	**0.7**	**1.0**																									
WUE	0.0	-0.1	-0.1	0.1	0.0	0.0	0.1	-0.2	**1.0**																								
IWUE	-0.3	0.0	-0.4	-0.1	0.1	-0.1	0.0	-0.3	**0.7**	**1.0**																							
ϕPSII	-0.1	0.2	0.1	**1.0**	0.3	**0.9**	**0.9**	**0.8**	0.1	-0.1	**1.0**																						
gs	-0.1	-0.1	0.1	**0.6**	**0.4**	**0.7**	**0.6**	**0.7**	-0.1	-0.1	**0.6**	**1.0**																					
g_m	-0.3	-0.3	0.0	**0.4**	0.2	**0.5**	0.4	**0.5**	0.2	0.1	**0.5**	**0.6**	**1.0**																				
V_{cmax}	-0.2	-0.2	0.0	**0.8**	**0.5**	**0.9**	**0.8**	**0.8**	0.0	0.0	**0.8**	**0.8**	**0.7**	**1.0**																			
J_{max}	-0.3	-0.2	0.0	**0.7**	**0.5**	**0.8**	**0.8**	**0.8**	**0.0**	0.0	**0.8**	**0.8**	**0.7**	**1.0**	**1.0**																		
J_{max}/V_{cmax}	**-0.5**	0.2	**-0.6**	0.0	0.0	0.1	0.3	0.1	-0.1	0.1	0.1	0.1	0.0	0.1	0.3	**1.0**																	
P_r	-0.2	-0.2	0.0	**0.7**	0.4	**0.8**	**0.7**	**0.8**	0.1	0.0	**0.8**	**0.7**	**0.7**	**0.9**	**0.9**	0.1	**1.0**																
P_b	-0.1	0.0	0.0	**0.8**	0.3	**0.9**	**0.7**	**0.8**	0.0	-0.1	**0.8**	**0.8**	**0.7**	**0.9**	**0.9**	0.1	**0.9**	**1.0**															
P_l	**-0.4**	**-0.5**	-0.2	0.2	0.1	0.2	0.3	0.2	0.1	0.1	0.3	0.2	**0.5**	**0.5**	**0.5**	0.3	**0.5**	0.4	**1.0**														
P_s	0.2	0.2	0.0	**-0.7**	-0.3	**-0.8**	**-0.7**	**-0.7**	-0.1	0.0	**-0.7**	**-0.7**	**-0.7**	**-0.9**	**-0.9**	-0.1	**-1.0**	**-1.0**	**-0.6**	**1.0**													
SLA	-0.3	**-0.4**	0.0	0.2	0.2	0.2	0.4	0.0	0.3	0.1	0.3	0.0	0.3	0.3	0.4	0.4	0.3	0.1	**0.7**	-0.3	**1.0**												
LMA	0.3	0.4	0.0	-0.1	-0.1	-0.1	-0.3	0.0	-0.3	-0.2	-0.2	0.0	-0.3	-0.3	-0.3	-0.3	-0.2	0.0	**-0.6**	0.2	**-1.0**	**1.0**											
SL	-0.1	0.0	-0.2	**-0.5**	**-0.6**	**-0.8**	**-0.6**	**-0.7**	0.2	0.2	**-0.5**	**-0.8**	-0.3	**-0.6**	**-0.6**	0.0	**-0.6**	**-0.6**	-0.1	**0.5**	0.0	0.0	**1.0**										
ML	**0.5**	0.3	0.3	0.3	0.2	**0.4**	0.3	0.4	-0.3	-0.3	0.2	0.3	-0.3	0.1	0.0	-0.2	0.1	0.2	-0.3	-0.1	-0.3	0.3	**-0.7**	**1.0**									
BL	-0.4	-0.4	-0.1	0.4	**0.5**	**0.5**	**0.6**	**0.5**	0.1	0.1	**0.5**	**0.7**	**0.8**	**0.8**	**0.8**	0.2	**0.6**	**0.6**	**0.5**	**-0.6**	0.4	-0.3	**-0.5**	-0.2	**1.0**								
EUCa	-0.2	-0.4	0.2	**0.6**	**0.5**	**0.7**	**0.8**	**0.6**	0.1	0.0	**0.7**	**0.4**	**0.5**	**0.8**	**0.8**	0.0	**0.7**	**0.6**	**0.5**	**-0.7**	**0.6**	**-0.5**	**-0.5**	0.0	**0.7**	**1.0**							
EUMg	**-0.6**	0.2	**-0.5**	0.1	-0.3	0.0	0.1	0.1	-0.1	0.0	0.2	-0.1	0.1	0.0	0.1	**0.7**	0.1	0.1	**0.5**	-0.1	**0.5**	**-0.5**	0.2	-0.3	0.1	0.0	**1.0**						
EUK	**-0.5**	0.0	-0.2	0.3	-0.1	0.1	0.2	0.1	0.3	0.2	0.3	0.0	0.4	0.3	0.4	0.3	0.4	0.3	**0.4**	**-0.4**	**0.4**	**-0.4**	0.3	**-0.5**	0.3	0.2	**0.5**	**1.0**					
EU N	-0.2	-0.1	0.1	**0.8**	**0.5**	**0.8**	**0.7**	**0.7**	0.2	0.1	**0.8**	0.4	**0.5**	**0.8**	**0.8**	0.1	**0.8**	**0.7**	**0.4**	**-0.8**	**0.5**	-0.4	**-0.5**	0.1	**0.5**	**0.9**	0.2	**0.4**	**1.0**				
EUP	-0.1	-0.3	0.3	0.3	0.0	0.2	0.2	0.1	0.2	0.0	0.3	0.0	**0.4**	0.3	0.3	0.1	0.2	0.2	**0.5**	-0.3	**0.7**	**-0.7**	0.1	**-0.5**	0.4	**0.5**	0.4	**0.7**	**0.5**	**1.0**			
EUFe	-0.2	0.0	0.0	**0.6**	**0.5**	**0.6**	**0.7**	**0.4**	0.2	0.1	**0.7**	0.1	0.3	**0.6**	**0.6**	0.2	**0.5**	**0.5**	**0.4**	**-0.5**	**0.6**	**-0.6**	-0.3	0.0	**0.4**	**0.8**	0.3	**0.4**	**0.9**	**0.6**	**1.0**		
EUZn	-0.3	**-0.4**	0.0	**0.4**	**0.5**	**0.5**	**0.6**	0.3	0.1	0.1	**0.5**	0.1	0.3	**0.6**	**0.7**	0.2	**0.5**	0.4	**0.6**	**-0.5**	**0.7**	**-0.6**	-0.3	-0.2	**0.5**	**0.9**	0.2	0.2	**0.8**	**0.5**	**0.8**	**1.0**	
EUMn	-0.3	-0.3	-0.1	**0.6**	**0.6**	**0.7**	**0.9**	**0.6**	0.2	0.3	**0.7**	**0.7**	**0.5**	**0.9**	**0.9**	0.2	**0.8**	**0.7**	**0.4**	**-0.8**	**0.4**	-0.3	**-0.6**	0.1	**0.7**	**0.8**	0.0	0.2	**0.8**	0.2	**0.6**	**0.7**	**1.0**
EI(%)	-0.1	**0.5**	-0.4	-0.3	**-0.5**	**-0.5**	**-0.5**	-0.4	0.0	0.2	-0.4	-0.3	**-0.6**	**-0.6**	**-0.6**	0.1	**-0.5**	-0.4	**-0.5**	**0.5**	**-0.6**	**0.5**	**0.4**	0.1	**-0.7**	**-0.7**	0.1	-0.1	**-0.5**	**-0.6**	**-0.5**	**-0.6**	**-0.5**	**1.0**

Bold values represent significant correlations ($p < 0.05$).

Table 4: Pearson correlation (r) among photosynthetic parameters in tree species submitted to moderate shade (MS).

VARIABLES	R_d	α	LCP	A_{max}	LSP	ETR	R_P	E	WUE	IWUE	ϕPSII	g_s	g_m	V_{cmax}	J_{max}	J_{max}/V_{cmax}	P_r	P_b	P_i	P_s	SLA	LMA	SL	ML	BL	EUCa	EUMg	EUK	EU N	EUP	EUFe	EUZn	EUMn	EI(%)
R_d	**1.0**																																
α	0.0	**1.0**																															
LCP	**0.6**	-0.1	**1.0**																														
A_{max}	0.1	0.0	0.3	**1.0**																													
LSP	-0.1	0.2	0.4	**0.6**	**1.0**																												
ETR	0.2	-0.2	**0.5**	**0.9**	**0.5**	**1.0**																											
R_P	0.0	-0.4	0.3	**0.8**	**0.5**	**0.9**	**1.0**																										
E	0.0	-0.1	**0.5**	**0.8**	**0.6**	**0.8**	**0.8**	**1.0**																									
WUE	-0.3	-0.1	**-0.5**	-0.1	-0.2	-0.1	0.0	**-0.5**	**1.0**																								
IWUE	**-0.4**	0.0	-0.3	0.0	-0.1	0.0	0.1	-0.2	**0.7**	**1.0**																							
ϕPSII	0.0	-0.2	0.3	**0.9**	**0.6**	**0.9**	**0.9**	**0.8**	0.0	0.1	**1.0**																						
gs	0.0	**-0.4**	**0.5**	**0.6**	**0.6**	**0.8**	**0.8**	**0.7**	-0.2	-0.2	**0.7**	**1.0**																					
g_m	0.0	-0.3	0.2	0.3	0.3	0.4	**0.5**	**0.6**	-0.3	-0.4	**0.4**	**0.6**	**1.0**																				
V_{cmax}	-0.2	-0.1	0.2	**0.6**	0.3	**0.6**	**0.7**	**0.6**	0.1	0.2	**0.7**	**0.6**	**0.5**	**1.0**																			
J_{max}	-0.1	-0.2	0.3	**0.6**	**0.4**	**0.7**	**0.7**	**0.7**	0.0	0.1	**0.8**	**0.7**	**0.5**	**0.9**	**1.0**																		
J_{max}/V_{cmax}	0.3	-0.2	0.3	-0.1	0.1	0.0	-0.1	-0.1	-0.2	-0.3	-0.1	0.1	-0.3	**-0.5**	-0.3	**1.0**																	
P_r	-0.2	-0.3	0.2	0.4	**0.5**	**0.5**	**0.6**	0.4	0.0	0.1	**0.5**	**0.6**	**0.6**	**0.6**	**0.6**	-0.2	**1.0**																
P_b	-0.2	-0.4	0.3	0.3	**0.5**	**0.4**	**0.5**	0.4	0.0	-0.1	**0.5**	**0.6**	**0.5**	0.3	**0.5**	0.2	**0.9**	**1.0**															
P_i	-0.1	-0.1	0.1	0.1	**0.5**	0.1	0.3	0.2	0.0	0.0	0.2	**0.4**	0.3	0.1	0.1	0.0	0.4	0.4	**1.0**														
P_s	0.2	0.3	-0.2	-0.3	**-0.6**	**-0.4**	**-0.6**	-0.4	0.0	-0.1	**-0.5**	**-0.6**	**-0.6**	**-0.5**	**-0.5**	0.1	**-1.0**	**-0.9**	**-0.6**	**1.0**												
SLA	-0.3	**-0.5**	-0.1	-0.2	0.0	-0.1	0.1	-0.1	0.1	-0.3	-0.1	0.2	**0.4**	0.0	0.1	0.1	0.4	**0.5**	0.2	**-0.4**	**1.0**											
LMA	0.2	**0.4**	0.2	0.2	0.1	0.1	-0.1	0.1	-0.2	0.3	0.1	-0.1	-0.4	-0.1	-0.1	0.0	-0.3	-0.4	-0.1	0.3	**-1.0**	**1.0**										
SL	0.1	0.3	-0.3	-0.4	**-0.5**	**-0.5**	**-0.5**	-0.4	0.0	-0.1	**-0.5**	**-0.7**	0.0	-0.2	-0.3	-0.4	-0.3	**-0.5**	**-0.5**	**0.4**	0.0	-0.1	**1.0**									
ML	0.2	0.0	0.4	0.3	0.1	0.4	0.2	0.1	0.1	0.1	0.2	0.2	**-0.6**	0.0	0.1	**0.5**	-0.2	-0.1	-0.1	0.2	-0.3	0.2	-0.3	**1.0**								
BL	-0.2	-0.2	-0.2	-0.1	0.2	-0.1	0.1	0.1	-0.1	0.0	0.0	0.2	**0.6**	0.1	0.0	-0.3	**0.4**	0.4	0.4	**-0.5**	0.3	-0.2	-0.2	**-0.8**	**1.0**							
EUCa	0.1	-0.1	0.3	**0.5**	0.2	**0.5**	**0.5**	**0.6**	-0.3	**-0.5**	**0.5**	**0.5**	**0.6**	**0.4**	**0.5**	-0.1	0.3	0.3	0.0	-0.3	0.3	-0.4	0.1	0.1	-0.2	**1.0**						
EUMg	-0.3	-0.2	-0.1	0.2	-0.1	0.3	**0.4**	0.2	0.2	0.1	0.3	0.3	0.3	**0.6**	**0.5**	-0.3	0.3	0.1	0.1	-0.2	0.3	**-0.5**	0.0	0.0	0.0	0.3	**1.0**					
EUK	**-0.5**	0.1	-0.2	0.3	0.4	0.3	**0.4**	0.3	**0.4**	**0.6**	0.4	0.3	0.1	**0.6**	**0.5**	-0.4	**0.4**	0.3	0.1	-0.4	-0.1	0.1	-0.3	0.0	0.2	-0.1	0.4	**1.0**				
EU N	-0.2	-0.3	0.0	0.3	**0.5**	0.3	**0.5**	0.3	0.2	0.0	0.4	**0.5**	0.3	0.2	0.3	0.2	**0.6**	**0.7**	0.3	**-0.6**	**0.6**	**-0.6**	**-0.5**	0.0	0.2	0.3	0.2	**0.4**	**1.0**			
EUP	-0.4	0.1	-0.1	**0.5**	0.4	**0.5**	**0.5**	**0.4**	0.2	0.3	**0.5**	**0.4**	**0.4**	**0.8**	**0.7**	**-0.6**	**0.7**	**0.5**	0.1	**-0.6**	0.1	-0.2	-0.1	-0.1	0.2	0.4	**0.5**	**0.8**	0.3	**1.0**		
EUFe	-0.1	-0.2	0.2	**0.5**	0.3	**0.6**	**0.6**	**0.6**	-0.1	-0.2	**0.6**	**0.6**	**0.5**	**0.7**	**0.7**	-0.2	**0.5**	**0.4**	0.0	**-0.4**	0.4	**-0.5**	0.0	0.2	-0.2	**0.9**	**0.6**	0.2	**0.4**	**0.6**	**1.0**	
EUZn	-0.3	**-0.4**	-0.1	-0.1	0.2	-0.1	0.1	0.1	-0.2	-0.4	0.0	0.2	**0.5**	-0.1	0.0	0.2	0.4	**0.5**	0.3	**-0.4**	**0.8**	**-0.7**	0.0	-0.3	0.4	0.4	0.1	-0.3	**0.4**	0.0	0.3	**1.0**	
EUMn	0.0	-0.2	0.1	0.0	0.3	0.1	0.2	0.1	0.0	-0.1	0.1	0.2	-0.1	-0.4	-0.2	**0.7**	0.2	**0.5**	0.4	-0.3	0.3	-0.3	**-0.4**	0.2	0.0	0.0	-0.2	-0.1	**0.6**	-0.3	0.0	**0.5**	**1.0**
EI(%)	-0.1	-0.2	0.3	**0.5**	0.2	**0.6**	**0.6**	**0.4**	0.1	0.4	**0.6**	**0.5**	0.2	**0.8**	**0.8**	-0.1	**0.5**	0.4	-0.2	-0.4	-0.1	0.0	-0.3	0.3	-0.1	0.2	**0.5**	**0.6**	0.3	**0.7**	**0.5**	-0.2	-0.2	**1.0**

Bold values represent significant correlations ($p < 0.05$).

Table 5: Pearson correlation (r) among photosynthetic parameters in tree species submitted to deep shade (DS).

VARIABLES	R_d	α	LCP	A_{max}	LSP	ETR	R_P	E	WUE	IWUE	ϕPSII	g_s	g_m	V_{cmax}	J_{max}	J_{max}/V_{cmax}	P_r	P_b	P_l	P_s	SLA	LMA	SL	ML	BL	EUCa	EUMg	EUK	EU N	EUP	EUFe	EUZn	EUMn	EI(%)
R_d	**1.0**																																
α	-0.1	**1.0**																															
LCP	-0.2	0.2	**1.0**																														
A_{max}	0.1	0.2	0.5	**1.0**																													
LSP	0.1	0.0	0.2	**0.7**	**1.0**																												
ETR	0.0	-0.1	**0.5**	**0.8**	**0.7**	**1.0**																											
R_P	0.1	-0.3	0.3	**0.6**	**0.6**	**0.8**	**1.0**																										
E	-0.1	0.3	0.3	**0.6**	**0.4**	**0.5**	0.4	**1.0**																									
WUE	-0.1	-0.3	0.1	0.0	0.2	0.4	**0.5**	-0.4	**1.0**																								
IWUE	0.1	-0.1	0.1	0.0	0.4	0.3	0.4	-0.4	**0.9**	**1.0**																							
ϕPSII	0.0	-0.2	0.4	**0.8**	**0.7**	**0.9**	**0.9**	**0.5**	0.4	0.3	**1.0**																						
gs	-0.2	0.2	0.1	0.1	0.0	0.2	0.2	**0.5**	-0.2	-0.4	0.2	**1.0**																					
g_m	**-0.6**	-0.1	**0.5**	0.1	0.1	**0.5**	0.4	0.2	0.4	0.2	0.4	0.3	**1.0**																				
V_{cmax}	-0.4	0.0	0.4	0.3	0.4	**0.6**	**0.5**	**0.5**	0.2	0.2	**0.5**	**0.6**	**0.9**	**1.0**																			
J_{max}	-0.3	0.0	**0.6**	**0.5**	0.4	**0.7**	**0.6**	**0.5**	0.3	0.3	**0.6**	0.4	**0.8**	**0.9**	**1.0**																		
J_{max}/V_{cmax}	0.2	0.0	**0.5**	0.3	0.0	0.1	0.1	-0.1	0.2	0.2	0.2	**-0.5**	-0.3	-0.3	0.0	**1.0**																	
P_r	-0.2	0.1	0.2	0.2	**0.5**	**0.6**	0.4	0.3	0.2	0.3	0.4	**0.5**	**0.7**	**0.9**	**0.7**	**-0.6**	**1.0**																
P_b	-0.2	0.1	**0.5**	0.4	**0.6**	**0.7**	**0.5**	0.4	0.3	0.4	**0.5**	0.4	**0.7**	**0.9**	**0.9**	-0.2	**0.9**	**1.0**															
P_l	0.2	-0.1	**0.5**	**0.6**	**0.7**	**0.7**	**0.6**	0.3	**0.5**	**0.5**	**0.7**	0.0	0.2	0.4	**0.6**	0.3	0.3	**0.5**	**1.0**														
P_s	0.1	0.0	**-0.4**	**-0.5**	**-0.7**	**-0.8**	**-0.6**	-0.4	-0.4	**-0.5**	**-0.6**	-0.3	**-0.6**	**-0.8**	**-0.8**	0.2	**-0.8**	**-0.9**	**-0.8**	**1.0**													
SLA	-0.4	0.0	-0.1	-0.2	**-0.5**	-0.2	-0.4	-0.1	-0.2	-0.4	-0.3	0.2	0.4	0.2	0.1	-0.4	0.2	0.0	-0.4	0.1	**1.0**												
LMA	0.2	0.0	0.0	0.1	0.4	0.2	0.3	0.2	0.0	0.2	0.2	0.0	-0.3	-0.2	-0.1	0.2	-0.1	-0.1	0.2	0.0	**-0.9**	**1.0**											
SL	-0.1	-0.3	0.1	0.1	0.0	0.0	0.1	**-0.5**	0.4	0.3	0.2	**-0.7**	0.1	-0.3	-0.2	**0.5**	-0.4	-0.3	0.2	0.1	0.1	-0.2	**1.0**										
ML	0.3	0.3	0.0	0.2	0.1	-0.1	-0.1	**0.5**	**-0.5**	-0.4	-0.1	0.3	**-0.6**	-0.2	-0.1	0.2	-0.2	-0.2	0.0	0.1	-0.4	0.4	**-0.6**	**1.0**									
BL	-0.1	0.2	0.0	-0.3	-0.1	0.0	-0.1	0.3	-0.2	-0.2	-0.1	**0.7**	0.3	**0.5**	0.3	**-0.7**	**0.6**	**0.5**	-0.3	-0.2	0.1	0.0	**-0.9**	0.1	**1.0**								
EUCa	-0.2	-0.1	0.1	0.4	0.0	0.1	0.2	-0.2	0.0	-0.2	0.3	-0.2	0.2	0.0	0.0	0.2	-0.1	-0.1	0.0	0.1	0.4	**-0.5**	**0.6**	-0.4	**-0.5**	**1.0**							
EUMg	-0.4	0.1	0.2	0.4	0.2	**0.5**	0.3	0.3	0.1	-0.1	0.4	0.3	**0.7**	**0.7**	**0.6**	-0.3	**0.6**	**0.6**	0.2	**-0.5**	**0.6**	**-0.5**	0.0	**-0.4**	0.2	**0.5**	**1.0**						
EUK	0.3	0.3	0.1	0.4	0.3	0.4	0.3	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.2	-0.2	**0.4**	**0.4**	0.2	-0.4	-0.1	-0.1	-0.1	-0.2	0.2	0.3	0.4	**1.0**					
EU N	0.1	0.4	0.4	**0.8**	0.4	**0.6**	0.3	**0.5**	-0.1	-0.2	**0.5**	0.2	0.1	0.3	0.3	0.1	0.2	0.3	0.4	-0.4	0.1	-0.3	0.1	0.0	-0.1	**0.5**	**0.5**	**0.6**	**1.0**				
EUP	-0.1	0.4	0.0	0.1	-0.2	-0.1	-0.3	0.2	**-0.5**	**-0.5**	-0.2	0.4	0.1	0.1	-0.1	-0.4	0.2	0.1	-0.3	0.1	**0.5**	**-0.5**	-0.3	-0.1	0.4	0.4	0.4	**0.5**	**0.5**	**1.0**			
EUFe	-0.3	0.1	0.2	0.4	0.3	**0.5**	0.2	-0.1	0.2	0.2	0.4	0.0	0.4	0.4	0.3	-0.2	**0.5**	**0.5**	0.2	**-0.5**	0.4	-0.4	0.2	**-0.5**	0.0	**0.6**	**0.7**	**0.6**	**0.5**	0.4	**1.0**		
EUZn	-0.3	0.2	0.0	0.0	-0.3	-0.2	-0.3	0.4	**-0.6**	**-0.8**	-0.2	**0.6**	0.1	0.2	0.0	-0.4	0.1	0.0	-0.4	0.2	**0.6**	-0.4	**-0.5**	0.2	**0.5**	0.1	0.3	-0.1	0.3	**0.7**	0.0	**1.0**	
EUMn	0.2	0.1	**0.4**	**0.9**	**0.5**	**0.7**	**0.6**	0.4	0.1	0.2	**0.8**	0.0	0.2	0.4	**0.5**	0.3	0.3	**0.5**	**0.6**	**-0.5**	-0.1	-0.1	0.2	0.0	-0.2	**0.6**	0.4	**0.7**	**0.8**	0.2	**0.6**	-0.1	**1.0**
EI(%)	-0.4	-0.1	**-0.5**	**-0.5**	-0.4	-0.4	-0.2	-0.4	0.0	-0.2	-0.3	0.1	0.2	0.0	-0.2	**-0.5**	0.1	-0.2	**-0.5**	0.3	**0.5**	-0.3	0.0	**-0.5**	0.2	0.3	0.3	0.1	-0.3	0.3	0.3	0.3	-0.4	**1.0**

Bold values represent significant correlations ($p < 0.05$).