Brazilian Journal of Botany (2019) 42:239–247
https://doi.org/10.1007/s40415-019-00533-2

ORIGINAL ARTICLE

Fluorescence parameters among leaf photosynthesis-related traits are the best proxies for CO_2 assimilation in Central Amazon trees

Victor Alexandre Hardt Ferreira dos Santos[1] · **Bruce Walker Nelson**[1] ·
João Victor Figueiredo Cardoso Rodrigues[2] · **Maquelle Neves Garcia**[1] · **João Vitor Barbosa Ceron**[1] ·
Marciel José Ferreira[3]

Received: 12 December 2018 / Accepted: 2 May 2019 / Published online: 21 May 2019

Abstract

We investigated whether photosynthesis along the vertical profile of tropical forest can be represented by JIP-test parameters derived from the fluorescence induction curve instead of more commonly utilized traits. Photosynthesis-related traits were measured during four seasons in 45 trees along a vertical forest profile in the Central Amazon, and their combined (interspecific) correlations with canopy openness and P_{Nmax} (light-saturated photosynthesis) were examined. P_{Nmax}, canopy openness, the efficiency of electron transport in PSI (IP-phase and $\mathrm{RE}_0/\mathrm{ET}_0$), leaf mass per area and leaf phosphorus content had strong positive correlations ($r > 0.6$). JIP-test parameters related to the final steps of electron transport (IP-phase, $\mathrm{RE}_0/\mathrm{ET}_0$ and $\mathrm{PI}_{\mathrm{total}}$) were good predictors of P_{Nmax} during all seasons, whereas stomatal conductance had the strongest correlation with P_{Nmax} during the dry season. The JIP-test parameters related to PSII efficiency ($\mathrm{PI}_{\mathrm{ABS}}$ and F_V/F_M) were poorly associated with P_{Nmax}. Therefore, the P_{Nmax} variation along the vertical profile of Central Amazonian forest can be represented by JIP-test parameters, especially those related to the final steps of the electron transport chain.

Keywords Chlorophyll *a* fluorescence · JIP-test · Photosynthesis · Photosystem I · Tropical forest

1 Introduction

The diversity and productivity of complex ecosystems, such as tropical forests, are associated with the supply of resources and their efficient use by plant species (Valladares and Niinemets 2008; Ishii et al. 2013; Girardin et al. 2016). Along the tropical forest vertical profile, vegetation structure and composition lead to heterogeneity in resource availability. For example, light intensity is reduced exponentially from the canopy top to the understory (Castro 2000; Kenzo et al. 2015). Only 1–2% of the irradiance available at the top of the canopy is available in the understory layer (Chazdon and Fetcher 1984; Nicotra et al. 1999; Kenzo et al. 2015). Adapted genotypes or acclimated phenotypes can cope with low irradiance in the understory by mechanisms to efficiently intercept, absorb and utilize this resource in the photosynthetic process and thus allow a positive carbon balance (Valladares and Niinemets 2008). On the other hand, the damage caused by energy excitation excess in the photosynthetic apparatus due to the very high irradiance at the top canopy layer, which can exceed 2600 μmol m^{-2} s^{-1}, is attenuated by tolerance mechanisms (Wright and Colley 1994; Demmig-Adams and Adams 2000; Kenzo et al. 2006).

There is evidence that the microclimate along a tropical forest vertical profile imposes a substantial variation in photosynthesis-related leaf traits (Carswell et al. 2000; Domingues et al. 2005; Kenzo et al. 2006; Kosugi et al. 2012; Weerasinghe et al. 2014; Kenzo et al. 2015). Reduced light availability in the understory favors leaf traits that maximize interception and absorption of radiant energy, targeting a positive carbon budget. These traits include: low

---

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40415-019-00533-2) contains supplementary material, which is available to authorized users.

✉ Marciel José Ferreira
mjf.ufam@gmail.com

[1] Environmental Dynamics Coordination, National Institute for Amazon Research, Manaus, Amazonas, Brazil

[2] Center for Distance Education, Federal University of Amazonas, Manaus, Amazonas, Brazil

[3] Department of Forest Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil

leaf mass to area ratio (Evans and Poorter 2001), altered concentration of photosynthetic pigments (Lichtenthaler and Babani 2004), nitrogen partitioning in the thylakoid pigment–protein complexes (Evans 1989; Kenzo et al. 2006), thylakoid stacking in the chloroplast ultrastructure (Boardman 1977) and a low-light compensation point (Craine and Reich 2005; Valladares and Niinemets 2008). High irradiance in the upper canopy favors a different suite of leaf traits that increase light-use efficiency. These include high rates of photosynthesis and leaf dark respiration (Weerasinghe et al. 2014; Kenzo et al. 2015), high palisade parenchyma thickness (Kenzo et al. 2006), and high leaf nitrogen and phosphorus contents (Kenzo et al. 2006; Kosugi et al. 2012; Weerasinghe et al. 2014; Kenzo et al. 2015).

Considering that light intensity is the main factor affecting plant establishment in tropical moist forest (Poorter and Arets 2003), an investigation of the integrity and functionality of the photosynthetic apparatus associated with electron transport efficiency of upper canopy and of understory tree species in tropical forest is essential to an understanding of their possibly disparate mechanisms of light harvesting and use. The traits related to electron transport efficiency can be accessed by the OJIP transient of chlorophyll *a* fluorescence induction parameters (Stirbet and Govindjee 2011) and thus improve our understanding of light harvesting and use along the tropical forest profile.

Light energy absorbed by photosynthetic pigments can be used by photochemistry, or it can be lost as heat or as re-emission by fluorescence. In dark-adapted leaves, a polyphasic transient in chlorophyll *a* fluorescence is observed after sudden saturating light exposure (Stirbet and Govindjee 2011; Stirbet et al. 2018). This polyphasic-induced fluorescence curve is the basis for the JIP-test, which extracts parameters associated with successive electron acceptor reductions along the electron transport chain in the thylakoids (Strasser et al. 1995, 2010; Stirbet and Govindjee 2011). Thus, both the light energy assimilated and the energy lost to fluorescence can be obtained, providing an indicator of photosynthetic performance (efficiency). The most widely used JIP-test parameters related to light-use efficiency are: F_V/F_M, representing the maximum quantum yield of primary PSII photochemistry; PI_{ABS}, a performance index for energy conservation from PSII to intersystem electron acceptors (further than quinone A) and PI_{total}, a performance index for energy conservation from PSII to the reduction in PSI end acceptors (Strasser et al. 2010; Stirbet et al. 2018).

The maximum quantum yield of primary PSII photochemistry (F_V/F_M) has been widely utilized in studies investigating response of light and CO_2-saturated photosynthesis to abiotic stresses (Kalaji et al. 2016; Stirbet et al. 2018). However, under drought or ozone stress, parameters that integrate more electron transport steps (e.g., PI_{ABS} and PI_{total}) are more sensitive than F_V/F_M (Bussotti et al. 2011; Campos et al. 2014; Kalaji et al. 2016). A body of evidence demonstrates a close association between potential (saturated) photosynthesis and the last steps of electron transport chain parameters accessed by the JIP-test under different stressors (*e.g., light and phosphorus availability*, Lin et al. 2009; Cunha et al. 2016; *ozone stress*, Cascio et al. 2010; Bussotti et al. 2011; *ozone and water stress*, Desotgiu et al. 2012).

Therefore, to extend the use of chlorophyll *a* fluorescence measurements to examine photosynthetic performance in plants, we ask if JIP-test parameters are more closely associated with light-saturated photosynthesis along the vertical profile of a tropical forest than the more commonly used traits. We hypothesize that the changes in light-saturated photosynthesis caused by the gradient of light intensity from the understory to the canopy can be well represented by fast kinetics fluorescence parameters.

2 Materials and methods

Site and sampling description – The study site is a Central Amazon *terra firme* forest on a low plateau with well-drained clay-loam Oxisol. All studied trees were within 200 m of the "K34" micrometeorological tower (2°36.5′S, 60°12.6′W), maintained by the LBA program (Large-Scale Biosphere–Atmosphere Experiment), 90 km north of Manaus, Brazil. The climate is characterized by average annual precipitation of 2400 mm with two dry months (< 100 mm mo^{-1}) in August and September and very little variation in average monthly air temperature (24.6–26.9 °C) seasonality (Araújo et al. 2002).

We compared the leaf traits of a group of 19 upper canopy trees and 26 understory trees (Online Resource 1). The upper canopy group included 16 species, while the understory group had 24. Forty-two percent of the understory trees were from taxa confined to understory during their entire life cycle. The rest were juveniles of mid- and upper-canopy taxa, included because they constitute a large portion of all understory stems. In each of the two environments, we examined leaf traits during four seasons in the year 2015: wet (April); wet/dry transition (July); dry (September) and dry/wet transition (November). All leaf traits were measured during the four seasons, except anatomical traits measured only in the wet season. For all measurements, we used leaves of similar conditions. All were fully expanded, mature and healthy. Upper and middle canopy leaves were accessed using the "K-34" micromet tower and two canopy walkways, each 30 m long and suspended 20–25 m above the ground. We accessed each understory tree from the ground or with a ladder.

Canopy openness and PPFD – Canopy openness was measured with hemispherical photographs from a Digital Plant Canopy Imager (CI-110, CID Bio-Science, Camas, WA, USA). These were taken above each measured leaf in the four seasons under a cloudless sky at dusk or dawn. PPFD was logged at 30-s intervals from sunrise to sunset for 5–7 days during each of the four seasonal field campaigns. Two PAR sensors (MQS-B/ULM-500 logger, Heinz Walz, Germany) were installed on the LBA tower, one at 52 m height (about 15 m above the surrounding trees) and one in the understory at 1.3 m height.

Leaf morpho-anatomical traits – We measured the thickness of the entire leaf (LT), of palisade parenchyma (PP) and of spongy parenchyma (SP). These traits were obtained only in the wet season field campaign, using three leaves from each tree. A section of the leaf middle was fixed in FAA (formalin, acetic acid and alcohol) then transferred to 70% ethanol, sectioned with a microtome (15–25 µm thickness) and photographed with a light microscope (Zeiss Axioskop 2 and Zeiss Axio Cam MRC; Zeiss, Jena, German). The 200 × micrographs were analyzed with ANAT QUANTI 2.0 software (Aguiar et al. 2007). Leaf mass per area (LMA), as dry leaf mass in grams at 65 °C per fresh leaf area in m^2, was obtained from ten leaf disks from each of three leaves per tree per season.

Chloroplast pigments – Chloroplast pigment concentrations were obtained for three leaves per tree in each of the four seasons. We macerated 0.1 g of fresh leaf in 10 ml of 80% acetone with $MgCO_3$ then immediately added 10 ml of 100% acetone. The extract was filtered and absorbance measured (Ultrospec 2100 Pro UV/visible, Amersham Biosciences, Cambridge, UK) at the wavelengths of 663, 645 and 480 nm (Lichtenthaler and Wellburn 1983). Following Hendry and Price (1993), we calculated chlorophyll *a* and *b* contents per unit of leaf area (µmol cm^{-2}) and their ratio (Chl *a*:*b*).

Leaf nitrogen and phosphorus – Nitrogen and phosphorus concentrations were obtained for three leaves per tree per season. Leaves were oven-dried at 65 °C to constant mass. For leaf *N*, 0.1 g of dry leaf sample was digested for 10 h in $H_2O_2 + H_2SO_4$ at gradually increasing temperature to 350 °C (Miyazawa et al. 1999). An aliquot of 25 ml of extract was used for total *N* by the Kjeldahl method of distillation and titration (Bremner 1996). Concerning leaf *P* concentration, 0.5 g of dry leaf sample was subjected to nitro-perchloric digestion and concentration was determined at 750 nm by spectrophotometry following Murphy and Riley (1962).

Chlorophyll a fluorescence – Fluorescence and photosynthesis measurements were made using attached leaves and branches. We used a portable fluorimeter (PEA, MK2-9600, Hansatech, Norfolk, UK) to measure chlorophyll *a* fluorescence, always between 08:00 and 10:00 h. Three leaves per tree per season were selected and kept in the dark for 30 min, sufficient for complete oxidation of the photosynthetic electron transport chain. This dark-adapted leaf was then exposed to saturating light of 3000 µmol m^{-2} s^{-1} at a wavelength of 650 nm during 1 s. The chlorophyll *a* polyphasic transient steps were recorded and the parameters calculated according to the JIP-test (Online Resource 2; Strasser et al. 1995, 1999; Tsimilli-Michael and Strasser 2008; Strasser et al. 2010): After light exposure, the photon flux is absorbed by the PSII antenna (ABS) and a part of the energy is trapped by PSII reaction centers (TR) causing the quinone A (Q_A) reduction; the Q_A^- electron is transported to intersystem electron acceptors (ET) and finally to PSI electron acceptors (RE). Using the JIP-test, we calculated the reaction center density ($\gamma_{RC}/(1-\gamma_{RC})$ or RC/ABS), maximum quantum yield of PSII (φP_0 or F_V/F_M), efficiency with which an electron moves further than quinone Q_A^- (ΨE_0 or ET_0/TR_0) and efficiency with which an electron from the intersystem electron carriers is transferred to reducing end electron acceptors at the PSI acceptor side (δR_0 or RE_0/ET_0). From these parameters, we derived the performance index (PI_{ABS}) and total performance index (PI_{total}):

$$PI_{ABS}=\left(\frac{\gamma RC}{1-\gamma RC}\right)\cdot\left(\frac{\varphi Po}{1-\varphi Po}\right)\cdot\left(\frac{\Psi Eo}{1-\Psi Eo}\right)$$

$$PI_{total}=PI_{ABS}\cdot\left(\frac{\delta Ro}{1-\delta Ro}\right)$$

Additionally, we derived the IP-phase, which reflects the PSI/PSII stoichiometry (Ceppi et al. 2012).

Photosynthetic leaf traits – Light-saturated net photosynthetic rate (P_{Nmax}) and stomatal conductance (g_s) were obtained from photosynthesis–irradiance curves (P_N-PPFD). Measurements were made on one leaf per tree per season, between 08:00 and 13:00 h, using an open-flow gas exchange system LI-6400XT (LI-COR, USA). The LI-COR 6400 chamber was adjusted to a flow rate of 400 µmol s^{-1}; 400 µmol mol^{-1} of CO_2 concentration, 21 mmol mol^{-1} of H_2O vapor concentration and 31 °C of leaf temperature. P_N-PPFD curves were obtained at eleven steps of PPFD: 2000, 1500, 1000, 750, 500, 250, 100, 75, 50, 25 and 0 µmol m^{-2} s^{-1} (Santos-Junior et al. 2013). P_{Nmax} and g_s were extracted at the saturating light value in each P_N-PPFD curve. Saturation occurred between 500 and 2000 µmol m^{-2} s^{-1}. No P_N curves showed decline even at 2000 µmol m^{-2} s^{-1}.

Statistical analysis – In the cases of non-normality and/or non-homogeneity, the data were log-transformed (Zar 1999). Associations between canopy openness and leaf traits (P_{Nmax}, g_{s}, morpho-anatomical traits, leaf *N* and *P* content and fluorescence parameters)—and between P_{Nmax} versus leaf traits—were obtained by Pearson's correlation for each season and for the annual average. All analyses were performed using the statistical program R version 3.5.1 (R Core Team 2018).

3 Results

Canopy openness and irradiance availability – As expected, canopy openness increased exponentially along vertical forest profile and the PPFD difference between understory and the upper canopy was remarkable (Fig. 1). During the cloudy wet season, upper canopy daytime PPFD was typically in the range 150–750 µmol m^{-2} s^{-1} and only 14% of daytime records exceeded 1000 µmol m^{-2} s^{-1}. In the dry season, 62% of daytime records exceeded this threshold. Transition seasons were between these two extremes, with 50% and 46% of daytime records above 1000 µmol m^{-2} s^{-1} in the wet-to-dry and the dry-to-wet transitions, respectively. Average understory daytime PPFD was less than 5% of upper canopy daytime irradiance across all four seasons. Over 75% of the daytime irradiance values recorded in the understory were below 25 µmol m^{-2} s^{-1} in all four seasons.

Leaf traits associated with canopy openness – The highest and most seasonally consistent correlations were between canopy openness and six leaf traits: P_{Nmax}, LMA, *P*, RE_0/ET_0, PI_{total} and IP-phase (Table 1). These were followed by leaf nitrogen which also had seasonally consistent correlation with canopy openness ($r = 0.42$–0.62). More moderate positive correlations with canopy openness were seen in three out of four seasons for stomatal conductance and RC/ABS. We found moderate positive correlations between canopy openness and leaf thickness, palisade parenchyma thickness and spongy mesophyll thickness. Total chlorophyll and the ratio Chl *a:b* had no significant relationship to canopy openness in any season, with one exception. Traits with moderate positive correlation to canopy openness but only in one or two seasons of the year were ET_0/TR_0 and PI_{ABS}. F_V/F_M had a moderate negative correlation with openness, and only for two out of four seasons.

Leaf traits associated with P_{Nmax} – P_{Nmax} was significantly correlated in all seasons with seven traits that were a mix of morpho-anatomical traits, stomatal conductance, fluorescence parameters and nutrient concentration. The degree of correlation was seasonal (Table 2). In the dry season, the best association was observed between P_{Nmax} and g_{s}, while LMA; the JIP-test parameters RE_0/ET_0, PI_{total} and IP-phase; and the two nutrients concentrations (*N* and *P*) were the traits best correlated with P_{Nmax} in the other seasons.

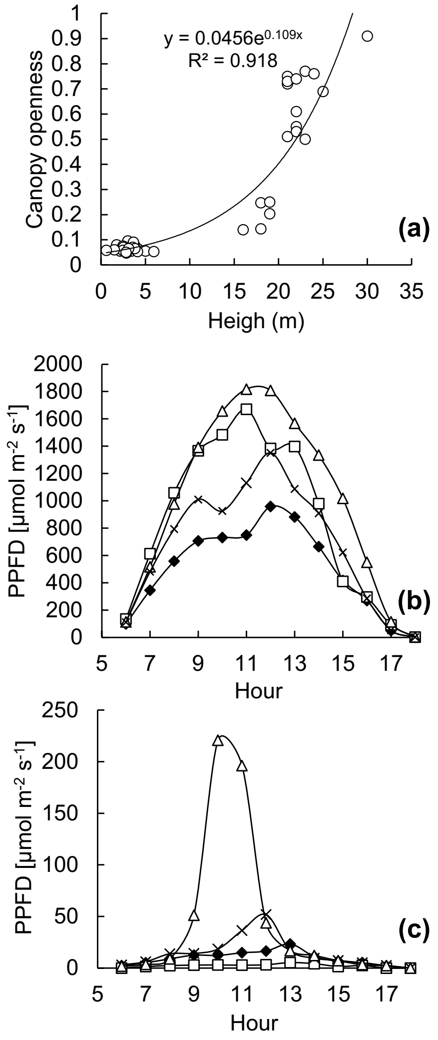

Fig. 1 Relationship between percent canopy openness and leaf height in Central Amazon forest (a), photosynthetic photon flux density as a function of time of day, measured above the upper canopy (b) and in the understory (c), from means of 5–7 days during each of the four seasons: wet (black diamond), wet/dry transition (white square), dry (white triangle) and dry/wet transition (cross)

When the data were lumped without distinguishing season, the best association was observed between P_{Nmax} and RE_0/ET_0. A strong correlation (> 0.60) was also observed for LMA, PI_{total}, IP-phase, *N* and *P*. Leaf and palisade parenchyma thicknesses were moderately correlated with P_{Nmax} in the lumped full year of data.

Table 1 Pearson's correlation between canopy openness and leaf traits in the four seasons and for average of the seasons

Canopy openness versus	Wet	Wet/dry	Dry	Dry/wet	Season's average
P_{Nmax}	**0.65**	**0.73**	**0.70**	**0.61**	**0.76**
LMA	**0.76**	**0.70**	**0.60**	**0.60**	**0.66**
g_s	**0.45**	0.13	**0.47**	**0.32**	0.26
Chl $a(b)$	−0.05	−0.18	−0.14	0.06	−0.11
Chl a:b	**0.41**	0.28	0.21	0.28	0.13
N	**0.62**	**0.42**	**0.44**	**0.45**	**0.49**
P	**0.74**	**0.70**	**0.60**	**0.64**	**0.78**
LT	–	–	–	–	**0.42**
PP	–	–	–	–	**0.55**
SP	–	–	–	–	**0.32**
F_V/F_M	−0.09	**−0.49**	−0.08	**−0.32**	**−0.35**
RC/ABS	**0.38**	0.20	**0.45**	**0.53**	**0.52**
$\mathrm{ET_0/TR_0}$	**0.40**	0.11	0.16	0.15	0.27
$\mathrm{PI_{ABS}}$	**0.38**	0.14	**0.34**	0.17	**0.39**
$\mathrm{RE_0/ET_0}$	**0.65**	**0.76**	**0.77**	**0.60**	**0.79**
$\mathrm{PI_{total}}$	**0.66**	**0.57**	**0.64**	**0.58**	**0.75**
IP-phase	**0.71**	**0.65**	**0.73**	**0.57**	**0.76**

The bolded coefficients are significant at $P < 0.05$

Table 2 Pearson's correlation between light-saturated photosynthesis and leaf traits in the four seasons and for average of the seasons

P_{Nmax} versus	Wet	Wet/dry	Dry	Dry/wet	Season's average
LMA	**0.56**	**0.49**	**0.45**	**0.62**	**0.63**
g_s	**0.65**	**0.38**	**0.82**	**0.72**	**0.57**
Chl $a(b)$	0.08	< 0.01	−0.01	**0.35**	0.15
Chl a:b	**0.41**	0.04	0.07	0.13	0.17
N	**0.67**	**0.38**	**0.38**	**0.52**	**0.60**
P	**0.74**	**0.39**	**0.61**	**0.77**	**0.75**
LT	–	–	–	–	**0.42**
PP	–	–	–	–	**0.53**
SP	–	–	–	–	0.25
F_V/F_M	0.04	**−0.47**	−0.19	−0.13	**−0.31**
RC/ABS	**0.60**	0.17	**0.39**	**0.64**	**0.45**
$\mathrm{ET_0/TR_0}$	**0.36**	0.05	0.21	0.20	**0.30**
$\mathrm{PI_{ABS}}$	**0.52**	0.02	0.22	**0.33**	**0.42**
$\mathrm{RE_0/ET_0}$	**0.57**	**0.63**	**0.76**	**0.76**	**0.82**
$\mathrm{PI_{total}}$	**0.68**	**0.46**	**0.64**	**0.73**	**0.78**
IP-phase	**0.60**	**0.55**	**0.73**	**0.72**	**0.79**

The bolded coefficients are significant at $P < 0.05$

4 Discussion

The novelty of this study lies, first, in reporting changes along the vertical light profile of a Central Amazon upland forest for a suite of morpho-anatomical and physiological leaf traits. Few investigations of this type have been published for the region (Carswell et al. 2000). Second, and more importantly, we show that, when data for several species are combined, photosynthetic rate variation along the vertical profile of tropical forest can be represented by certain traits derived from the chlorophyll *a* OJIP fluorescence transient, in the place of traditionally utilized traits. Specifically, IP-phase, $\mathrm{RE_0/ET_0}$ and $\mathrm{PI_{total}}$—all of which are JIP-test parameters related to the final steps of electron transport—were strongly correlated with P_{Nmax} during all seasons.

The ranges found here for leaf anatomical traits, chloroplast pigments, nitrogen and phosphorus, and photosynthetic leaf traits are consistent with those reported in other tropical forest tree species found in different parts of the vertical canopy profile (Kenzo et al. 2006; Cavaleri et al. 2010; Domingues et al. 2013; Kenzo et al. 2015). The range of F_V/F_M is also compatible with other surveys for tropical tree species (Krause et al. 2012). While few published values of JIP parameters are to be found for tropical trees, our data are within the ranges for tree species in general (Desotgiu et al. 2012).

Amazon forest leaf-level photosynthesis as explained by conventionally employed leaf traits – Leaf morphology and anatomy are known to change along the vertical light gradient of tropical forests (Kenzo et al. 2006; Cavaleri et al. 2010). Maximum attainable photosynthetic rate (P_{Nmax}) was here shown to be greater as canopy openness and morpho-anatomical leaf traits' values (LMA, LT, PP and SP) increased (Table 1). A high LMA as a consequence of a thicker leaf and thicker palisade parenchyma layers maximizes the utilization of the higher available irradiance of the upper canopy by increasing the number of mesophyll chloroplasts (Kenzo et al. 2004; Oguchi et al. 2005; Kenzo et al. 2006) and, consequently, increases P_{Nmax}. Therefore, the moderate associations between P_{Nmax} and morpho-anatomical traits are linked by efficient utilization of the light as canopy openness increases.

With few exceptions, neither total chlorophyll nor Chl a:b was related to canopy openness or photosynthesis (Tables 1, 2). The exceptions were weak associations of canopy openness and photosynthesis with Chl a:b during the wet season and between photosynthesis and total chlorophyll during the dry/wet transition season. Chl a:b ratio has been related to the size of the light-harvesting complex and, consequently, the fraction of energy absorbed by leaves (Niinemets 2010). During the wet season, light can be limiting to photosynthesis in tropical forests (Graham et al. 2003) and thus increasing the importance of efficient light harvesting along the vertical forest profile.

Photosynthesis had a moderate to a strong positive correlation with *N* and *P* leaf concentrations (Tables 1, 2). Higher nitrogen and phosphorous investments per unit of leaf area ensure higher maximum rates of photosynthesis in the upper canopy—as expected in a high resource environment—according to the leaf economic spectrum (Wright et al. 2004). Increasing photosynthesis under high irradiance has been related to the partitioning of *N* to enzymes of the Calvin–Benson cycle and other proteins involved in the photosynthetic process, while *P* is partitioned to inorganic phosphate and intermediate metabolites, such as Ribulose 1,5 bisphosphate (Evans 1989; Hidaka and Kitayama 2009, 2013). The stronger association between phosphorus and photosynthesis when compared with nitrogen (Table 2) can be related to phosphorus being derived from the geologic substrate and thus strongly limiting in highly weathered Amazonian soils (Vitousek and Sanford 1986; Quesada et al. 2012), whereas *N* is fixed from the atmosphere by biological processes.

Stomatal conductance was the trait most strongly correlated with leaf-level photosynthesis in the peak dry season month of September (Table 2). The September field campaign coincided with a strong drought across a large part of the Amazon Basin (Jiménez-Muñoz et al. 2016), set off by an El Niño-Southern Oscillation event. Particularly, a photosynthesis decrease in this extreme dry season, compared to a "normal" dry season 1 year later, was caused by stomatal closure at our Central Amazon site (Santos et al. 2018). Therefore, the strong association between P_{Nmax} and g_s during the dry season could be related to the unusually extreme water deficit of 2015.

JIP-test parameters related to PSII are poor predictors of P_{Nmax} – JIP-test parameters, except for F_V/F_M, increase together with canopy openness (Table 1), and a moderate-to-strong correlation was seen between P_{Nmax} and those JIP-test parameters that are related to the final steps of electron transport (PSI) during all seasons (Table 2). In contrast, we found a weak negative relationship between F_V/F_M (a PSII parameter) and canopy openness. This could be related to dynamic photoinhibition of PSII processes under high irradiance (Demmig-Adams and Adams 2000). Chlorophyll *a* fluorescence was recorded between 08:00 and 10:00 h when the irradiance reached values of 600–1600 μmol m^{-2} s^{-1}, enough to trigger photoprotective processes such as thermal dissipation on sun-exposed leaves (Demmig-Adams and Adams 2006; Desotgiu et al. 2012; Krause et al. 2012). Therefore, the negative correlation between F_V/F_M and canopy openness, and consequently photosynthesis, can arise from a reduction in energy trapping by PSII primary acceptors as a consequence of non-photochemical quenching. Unfortunately, a pre-dawn condition of F_V/F_M—after dark recovery—was not possible to measure, precluding a definitive conclusion about dynamic and permanent photoinhibition. In conditions of high irradiance exposure, some tropical tree species cannot recover the pre-dawn values of F_V/F_M, indicating a chronic photoinhibition (Castro et al. 1995; Ribeiro et al. 2005, Quevedo-Rojas et al. 2018). The PI_{ABS} index is a combination of three PSII parameters, all of which were poor predictors of photosynthesis. These are F_V/F_M, the total number of active PSII reaction centers per unit of absorption flux (RC/ABS), and the efficiency that an electron moves further than quinone Q_A^- (ET_0/TR_0). RC/ABS was moderate to weakly correlated with canopy openness and photosynthesis, while ET_0/TR_0 was correlated only in the wet season (Tables 1, 2). Therefore, the poor, and in some cases absent, correlation of PI_{ABS} Index with canopy openness and photosynthesis was a result of weak correlation by its components, principally F_V/F_M and ET_0/TR_0. However, it should be noted that a moderate correlation was observed between PI_{ABS} and photosynthesis in the wet season, provided by the RC/ABS contribution. Consistent with the close association between photosynthesis and Chl *a*:*b* during the wet season, the improved wet season association between P_{Nmax} and PI_{ABS} is further evidence of light limitation acting on the first PSII steps of photosynthesis (Graham et al. 2003). In an extensive survey of European forest stands (Pollastrini et al. 2016), PI_{ABS} was the most effective chlorophyll *a* fluorescence parameter to describe the photochemical properties of tree species and F_V/F_M was associated with light-saturated photosynthesis (clustered at first PCA axis) in broadleaved tree species.

JIP-test parameters related to the final steps of electron transport are good proxies of photosynthesis – Though P_{Nmax} was correlated with parameters at different stages of electron transport, it was most strongly correlated with the final stages of electron transport (IP-phase and RE_o/ET_o; Table 2). Thus, jointly with photosynthesis, the IP-phase and RE_o/ET_o were greater as canopy openness increased (Table 1). Under high irradiance levels, the PSI in sun leaves of tropical trees has energy dissipation mechanisms that protect it against photo-oxidative damage (Barth et al. 2001) and allow PSI to continue functioning at high efficiency. Moreover, the higher values of the IP-phase parameter detected in the upper canopy are an indirect indication of a higher PSI/PSII ratio (Schansker et al. 2003, 2005) as also observed in well-watered and nutrient-supplied plants (Oukarroum et al. 2009; Nikiforou and Manetas 2011; Ceppi et al. 2012). The IP-phase amplitude increases in leaves of tree canopies exposed to high irradiance in mixed temperate forest stands (Pollastrini et al. 2016, 2017). The sun leaves of *Fagus sylvatica* L. trees, for example, have a lower capacity to trap electrons at PSII (low F_V/F_M), but a great efficiency in reducing the PSI end electrons acceptors (RE_o/ET_o) when compared with shade leaves (Cascio et al. 2010; Desotgiu et al. 2012). Thus, in the well-illuminated upper canopy leaves, a higher PSI:PSII ratio combined with the occurrence of effective PSI protection mechanisms may increase the efficiency of electron transfer to the acceptors at the PSI side (RE_o/ET_o) and the overall efficiency index is increased (PI_{total}).

The reduction power produced in the electron transport chain in the thylakoid is crucial for the Calvin–Benson cycle intermediate reduction stages and, consequently, higher CO_2 assimilation (Sharkey et al. 2007). Furthermore, the PSI acceptor side can be limited by the activity of the Calvin–Benson cycle (Schansker et al. 2003). Therefore, the close association between light-saturated photosynthesis and the fluorescence parameters related to the end steps of electron transport arises from the feedback between high supply and demand of reduction power (NADPH) in a well-illuminated—high resource—environment. In plants submitted to nutrient and water limitation (Lin et al. 2009; Campos et al. 2014) and ozone stress (Desotgiu et al. 2012), RE_o/ET_o and PI_{total} are low, relative to well-supplied plants. This is related to reduced PSI (as derived from PSI/PSII stoichiometry), which reduces CO_2 assimilation.

The investigation of the photosynthetic apparatus function by the rapid and noninvasive JIP-test provides information about photosynthetic performance under various environmental conditions. We have here extended this to include the vertical light profile of a tropical forest. Moreover, we have demonstrated that JIP-test parameters related to the final steps of electron transport predict light-saturated photosynthesis as well as or better than the usually utilized traits.

Acknowledgements We thank the Federal University of Amazonas (UFAM), Brazil's National Institute for Amazon Research (INPA) and the Large-Scale Biosphere–Atmosphere Experiment in Amazonia Program (LBA) for logistic support; the GOAmazon project, funded jointly by the US Department of Energy (DOE, # DE. SC0008383), by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), and by the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM, # 062.00570/2014); the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a fellowship.

Author's contributions VAHFS and MJF designed the research. VAHFS, MNG and JVBC collected the data. VAHFS analyzed the data and wrote a draft of the manuscript. BWN and JVFCR revised the text. All authors contributed to the final version of the manuscript and approved submission.

Compliance with ethical standards

Conflict of interest None declared.

References

Aguiar TV, Sant'anna-Santos BF, Azevedo AA, Santos RF (2007) Anati quanti: software de análises quantitativas para estudos em anatomia vegetal. Planta Daninha 25:649–659

Araújo AC, Nobre AD, Kruijt B, Elbers JA, Dallarosa R, Stefani P, Randow C, Manzi AO, Culf AD, Gash JHC, Valentini R, Kabat P (2002) Comparative measurements of carbon dioxide fluxes from two nearby towers in a central Amazonian rainforest: the Manaus LBA site. J Geophys Res 107:80–90

Barth C, Krause GH, Winter K (2001) Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves. Plant Cell Environ 24:163–176

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. Annu Rev Plant Physiol 28:355–377

Bremner JM (1996) Nitrogen-total. In: Sparks DL (ed) Methods of soil analysis, part 3, chemical methods. American Society of Agronomy, Madison, WI, pp 1085–1121

Bussotti F, Desotgiu R, Cascio C, Pollastrini M, Gravano E, Gerosa G, Manes F (2011) Ozone stress in woody plants assessed with chlorophyll a fluorescence. A critical reassessment of existing data. Environ Exp Bot 73:19–30

Campos H, Trejo C, Valdiva P, Nava G, Martinez C, Ortega C (2014) Stomatal and non-stomatal limitations of bell pepper plants under water stress and re-watering: delayed restoration of growth and photosynthesis during recovery. Environ Exp Bot 98:56–64

Carswell FE, Meir P, Wandelli EV, Bonates LCM, Kruijt B, Barbosa EM, Nobre AD, Grace J, Jarvis PG (2000) Photosynthetic capacity in a central Amazonian rain forest. Tree Physiol 20:179–186

Cascio C, Schaub M, Novak K, Desotgiu R, Bussotti F, Strasser RJ (2010) Foliar responses to ozone of *Fagus sylvatica* L. seedlings grown in shaded and in full sunlight conditions. Environ Exp Bot 68:188–197

Castro F (2000) Light spectral composition in a tropical forest: measurements and model. Tree Physiol 20:49–56

Castro Y, Fetcher N, Fernández DS (1995) Chronic photoinhibition in seedlings of tropical trees. Physiol Plantarum 94:560–565

Cavaleri MA, Oberbauer SF, Clark DB, Clark DA, Ryan MG (2010) Height is more important than light in determining leaf morphology in a tropical forest. Ecology 91:1730–1739

Ceppi MG, Oukarroum A, Çiçek N, Strasser RJ, Schansker G (2012) The IP amplitude of the fluorescence rise OJIP is sensitive to changes in the photosystem I content of leaves: a study on plants exposed to magnesium and sulfate deficiencies, drought stress and salt stress. Physiol Plantarum 144:277–288

Chazdon RL, Fetcher N (1984) Photosynthetic light environments in a lowland tropical rain forest in Costa Rica. J Ecol 72:553–564

Craine JM, Reich PB (2005) Leaf-level light compensation points in shade-tolerant woody seedlings. New Phytol 166:710–713

Cunha HFV, Gonçalves JFC, Santos Junior UM, Ferreira MJ, Peixoto PHP (2016) Biomassa, trocas gasosas e aspectos nutricionais de plantas jovens de pau de balsa (*Ochroma pyramidale* (Cav. Ex Lamb.) Urb.) submetidas à fertilização fosfatada em ambientes contrastantes de irradiância. Sci For 44:215–230

Demmig-Adams B, Adams WW III (2000) Photosynthesis: harvesting sunlight safely. Nature 403:371–374

Demmig-Adams B, Adams WW III (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol 172:11–21

Desotgiu R, Pollastrini M, Cascio C, Gerosa G, Marzuoli R, Bussotti F (2012) Chlorophyll a fluorescence analysis along a vertical gradient of the crown in a poplar (Oxford clone) subjected to ozone and water stress. Tree Physiol 32:976–986

Domingues TF, Berry JA, Martinelli LA, Ometto JP, Ehleringer JR (2005) Parameterization of canopy structure and leaf-level gas exchange for an eastern Amazonian tropical rain forest (Tapajos National Forest, Para, Brazil). Earth Interact 9:1–23

Domingues TF, Martinelli LA, Ehleringer JR (2013) Seasonal patterns of leaf-level photosynthetic gas exchange in an eastern Amazonian rain forest. Plant Ecol Divers 7:189–203

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19

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

Girardin CA, Malhi Y, Doughty CE, Metcalfe DB, Meir P, Aguila-Pasquel J, Araujo-Murakami A, da Costa ACL, Silva-Espejo JE, Amézquita FF, Rowland L (2016) Seasonal trends of Amazonian rainforest phenology, net primary productivity, and carbon allocation. Global Biogeochem Cy 30:700–715

Graham EA, Mulkey SS, Kitajima K, Phillips NG, Wright SJ (2003) Cloud cover limits net CO_2 uptake and growth of a rainforest tree during tropical rainy seasons. Proc Natl Acad Sci USA 100:572–576

Hendry GAF, Price AH (1993) Stress indicators: chlorophylls and carotenoids. In: Hendry GAF, Grime JP (eds) Methods in comparative plant ecology. Chapman & Hall, London, pp 148–152

Hidaka A, Kitayama K (2009) Divergent patterns of photosynthetic phosphorus-use efficiency versus nitrogen-use efficiency of tree leaves along nutrient-availability gradients. J Ecol 97:984–991

Hidaka A, Kitayama K (2013) Relationship between photosynthetic phosphorus-use efficiency and foliar phosphorus fractions in tropical tree species. Ecol Evol 3:4872–4880

Ishii H, Azuma W, Nabeshima E (2013) The need for a canopy perspective to understand the importance of phenotypic plasticity for promoting species coexistence and light-use complementarity in forest ecosystems. Ecol Res 28:191–198

Jiménez-Muñoz JC, Mattar C, Barichivich J, Santamaría-Artigas A, Takahashi K, Malhi Y, Sobrino JA, van der Schrier G (2016) Record-breaking warming and extreme drought in the Amazon rainforest during the course of El Niño 2015–2016. Sci Rep UK 6:33130

Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Cetner MD, Lukasik I, Goltsev V, Ladle RJ (2016) Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiol Plant 38:102

Kenzo T, Ichie T, Yoneda R, Kitahashi Y, Watanabe Y, Ninomiya I, Koike T (2004) Interspecific variation of photosynthesis and leaf characteristics in canopy trees of five species of Dipterocarpaceae in a tropical rain forest. Tree Physiol 24:1187–1192

Kenzo T, Ichie T, Watanabe Y, Yoneda R, Ninomiya I, Koike T (2006) Changes in photosynthesis and leaf characteristics with tree height in five dipterocarp species in a tropical rain forest. Tree Physiol 26:865–873

Kenzo T, Inoue Y, Yoshimura M, Yamashita M, Tanaka-Oda A, Ichie T (2015) Height-related changes in leaf photosynthetic traits in diverse Bornean tropical rain forest trees. Oecologia 177:191–202

Kosugi Y, Takanashi S, Yokoyama N, Philip E, Kamakura M (2012) Vertical variation in leaf gas exchange parameters for a Southeast Asian tropical rainforest in Peninsular Malaysia. J Plant Res 125:735–748

Krause GH, Winter K, Matsubara S, Krause B, Janhs P, Virgo A, Aranda J, García M (2012) Photosynthesis, photoprotection, and growth of shade-tolerant tropical tree seedlings under full sunlight. Photosynth Res 113:273–285

Lichtenthaler HK, Babani F (2004) Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In: Papageorgiou GC, Govindjee (eds) Chlorophyll fluorescence: a signature of photosynthesis. Springer, Dordrecht, pp 713–736

Lichtenthaler HK, Wellburn AR (1983) Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc T 11:591–592

Lin ZH, Chen LS, Chen RB, Zhang FZ, Jiang HX, Tang N (2009) CO_2 assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase, carbohydrates and photosynthetic electron transport probed by the JIP-test, of tea leaves in response to phosphorus supply. BMC Plant Biol 9:43

Miyazawa M, Pavan MA, Muraoka T, Carmo CAFS, Mello WJ (1999) Análise química de tecidos vegetais. In: Silva FC (ed) Manual de Análise Química de Solos, Plantas e Fertilizantes. EMBRAPA, Brasília, pp 172–223

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Nicotra AB, Chazdon RL, Iriarte SV (1999) Spatial heterogeneity of light and woody seedling regeneration in tropical wet forests. Ecology 80:1908–1926

Niinemets Ü (2010) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. Ecol Res 25:693–714

Nikiforou C, Manetas Y (2011) Inherent nitrogen deficiency in *Pistacia lentiscus* preferentially affects photosystem I: a seasonal field study. Funct Plant Biol 38:848–855

Oguchi R, Hikosaka K, Hirose T (2005) Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. Plant Cell Environ 28:916–927

Oukarroum A, Schansker G, Strasser RJ (2009) Drought stress effects on photosystem I content and photosystem II thermotolerance analyzed using Chl *a* fluorescence kinetics in barley varieties differing in their drought tolerance. Physiol Plantarum 137:188–199

Pollastrini M, Holland V, Brüggemann W, Bruelheide H, Dănilă I, Jaroszewicz B, Valladares F, Bussotti F (2016) Taxonomic and ecological relevance of the chlorophyll *a* fluorescence signature of tree species in mixed European forests. New Phytol 212:51–65

Pollastrini M, Nogales AG, Benavides R, Bonal D, Finer L, Fotelli M, Gessler A, Grossiord C, Radoglou K, Strasser RJ, Bussotti F (2017) Tree diversity affects chlorophyll a fluorescence and other leaf traits of tree species in a boreal forest. Tree Physiol 37:199–208

Poorter L, Arets EJMM (2003) Light environment and tree strategies in a Bolivian tropical moist forest: an evaluation of the light partitioning hypothesis. Plant Ecol 166:295–306

Quesada CA, Phillips OL, Schwarz M et al (2012) Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. Biogeosciences 9:2203–2246

Quevedo-Rojas A, García-Núñez C, Jerez-Rico M, Jaimez R, Schwarzkopf T (2018) Leaf acclimation strategies to contrasting light conditions in saplings of different shade tolerance in a tropical cloud forest. Funct Plant Biol 45:968–982

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ribeiro RV, Souza GM, Oliveira RF, Machado EC (2005) Photosynthetic responses of tropical tree species from different successional groups under contrasting irradiance conditions. Braz J Bot 28:149–161

Santos-Junior UM, Gonçalves JFC, Fearnside PM (2013) Measuring the impact of flooding on Amazonian trees: photosynthetic response models for ten species flooded by hydroelectric dams. Trees Struct Funct 27:193–210

Santos VAHFD, Ferreira MJ, Rodrigues JVFC, Garcia MN, Ceron JVB, Nelson BW, Saleska SR (2018) Causes of reduced leaf-level photosynthesis during strong El Niño drought in a Central Amazon forest. Glob Change Biol 24:4266–4279

Schansker G, Srivastava A, Strasser RJ (2003) Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves. Funct Plant Biol 30:785–796

Schansker G, Tóth SZ, Strasser RJ (2005) Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP. Biochim Biophys Acta 1706:250–261

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C3 leaves. Plant Cell Environ 30:1035–1040

Stirbet A, Govindjee (2011) On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. J Photoch Photobio B 104:236–257

Stirbet A, Lazár D, Kromdijk J, Govindjee (2018) Chlorophyll a fluorescence induction: can just a one-second measurement be used to quantify abiotic stress responses? Photosynthetica. https://doi.org/10.1007/s11099-018-0770-3

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61:32–42

Strasser RJ, Srivastava A, Tsimilli-Michael M (1999) Screening the vitality and photosynthetic activity of plants by fluorescence transient. In: Behl RK, Punia MS, Lather BPS (eds) Crop improvement for food security. SSARM, Hisar, pp 72–115

Strasser RJ, Tsimilli-Michael M, Giang S, Goltsev V (2010) Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. Biochim Biophys Acta 1797:1313–1326

Tsimilli-Michael M, Strasser RJ (2008) In vivo assessment of plants' vitality: applications in detecting and evaluating the impact of mycorrhization on host plants. In: Varma A (ed) Mycorrhiza: state of the art. Genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics, 3rd edn. Springer, Dordrecht, pp 679–703

Valladares F, Niinemets Ü (2008) Shade tolerance, a key plant feature of complex nature and consequences. Annu Rev Ecol Evol S 39:237–257

Vitousek PM, Sanford RL (1986) Nutrient cycling in moist tropical forest. Ann Rev Ecol Syst 17:137–167

Weerasinghe LK, Creek D, Crous KY, Xiang S, Liddell MJ, Turnbull MH, Atkin OK (2014) Canopy position affects the relationships between leaf respiration and associated traits in a tropical rainforest in Far North Queensland. Tree Physiol 34:564–584

Wright SJ, Colley M (1994) Accessing the canopy: Phase I. United Nation Environmental Program, Nairobi, Kenya, Assessment of Biological Diversity and Microclimate of the Tropical Forest Canopy

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Nava ML, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821

Zar JH (1999) Biostatical analysis. Prentice Hall, New Jersey