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Mesophyll conductance variations in response to diurnal environmental factors in *Myrcia paivae* and *Minquartia guianensis* in Central Amazonia

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Abstract

Mesophyll conductance (g_m) is essential to determine accurate physiological parameters used to model photosynthesis in forest ecosystems. This study aimed to determine the effects of time of day on photosynthetic parameters, and to assess the effect of using either intercellular CO_2 concentration (C_i) or chloroplast CO_2 concentration (C_c), on maximum carboxylation velocity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), V_{cmax}. We used Amazonian saplings of *Myrcia paivae* and *Minquartia guianensis*. Photosynthetic parameters were measured using an infrared gas analyzer (IRGA); g_m was determined using both gas exchange and chlorophyll (Chl) *a* fluorescence and gas-exchange data alone. Leaf thickness (L_T) and specific leaf area (SLA) were also measured. Air temperature, relative humidity or understory light did not correlate with g_m and on average daily IRGA-fluorometer-determined g_m was 0.04 mol(CO_2) m^{-2} s^{-1} for *M. paivae* and 0.05 mol(CO_2) m^{-2} s^{-1} for *M. guianensis.* Stomatal conductance (g_s), g_m, electron transport rate (J_F), and light-saturated net photosynthetic rate (P_{Nmax}) were lower in the afternoon than in the morning. However, no effect of time of day was observed on V_{cmax}. L_T and SLA did not affect any of the examined parameters. IRGA-determined g_m was almost the double of the value obtained using the IRGA-fluorescence method. V_{cmax} values determined using C_c were about 25% higher than those obtained using C_i, which highlighted the importance of using C_c in V_{cmax} calculation. Decline in P_{Nmax} at the end of the afternoon reflected variations in g_s and g_m rather than changes in V_{cmax}. Diurnal variation in g_m appeared to be associated more with endogenous than with atmospheric factors.

Additional key words: chloroplast carbon dioxide concentration; electron transport rate; maximum carboxylation velocity of Rubisco; stomatal conductance.

Introduction

Among the factors determining net photosynthetic rate (P_N), g_s has received most attention and it is the best characterized (Kumar *et al.* 1999, Park and Furukawa 1999, Massacci *et al.* 2008). Another important component of photosynthesis is g_m. It can constrain photosynthesis to about the same extent as g_s does (Flexas *et al.* 2012), but its role as a key photosynthetic parameter was recognized only several years after the pioneer work of Gaastra (1959), in part, because the early instrumentation required was not available for most laboratories. Although photosynthesis is one of the most important physiological parameters, mathematical models that aim to predict the rate of carbon assimilation in forest ecosystems use V_{cmax} as the main parameter. This is

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Abbreviations: C_i − intercellular CO_2 concentration; C_c – chloroplast CO_2 concentration; Chl – chlorophyll; [CO_2] − CO_2 concentration; F_m − maximum chlorophyll fluorescence of a dark-adapted leaf; F_m'− maximum fluorescence of an illuminated leaf; F_s – steady-state fluorescence; g_m − mesophyll conductance; g_s − stomatal conductance; I_e − photosynthetically active radiation absorbed by a leaf; J_F – electron transport rate; $J_{F\text{-cal}}$ – J_F corrected; J_c – flow of electrons used for carboxylation of RuBP; J_o – flow of electrons used for oxygenation of RuBP; L_T − fresh leaf thickness; K_c – Michaelis constant of Rubisco for carboxylation; K_o – Michaelis constant of Rubisco for oxygenation; PAR – photosynthetically active radiation; P_N − net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; P_N/C_i − response of photosynthesis to intercellular CO_2 concentration; PSII – photosystem II; RH − air relative humidity; R_L – leaf respiration in the light; Rubisco – ribulose-1,5-bisphosphate carboxylase; RuBP – ribulose-1,5-bisphosphate; S^* − specificity factor of Rubisco; S − Rubisco specificity *in vitro* (2,560 mol mol^{-1}); SLA – specific leaf area; T_{air} – air temperature; V_{cmax} – maximum carboxylation velocity of Rubisco; α − leaf absorptance; Φ_{PSII} – quantum yield of photosystem II.

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because V_{cmax} depends on C_i rather than on C_a, which minimizes the effect of g_s.

Several studies have been conducted in the Amazon to determine photosynthetic parameters of Amazonian trees (*e.g*. Carswell *et al*. 2000, Domingues *et al*. 2007, Azevedo and Marenco 2012). However, to our knowledge in all these studies, V_{cmax} has been determined by using C_i, (apparent V_{cmax}) rather than chloroplast [CO_2], C_c. Simon *et al*. (2005) suggest that the accuracy to determine biochemical parameters of Amazonian trees need to be improved to avoid overestimation of predicted net assimilation. In a recent study, Keenan *et al.* (2010) concluded that photosynthetic responses to changes in soil water moisture can be only described accurately if g_m was included in the model so that C_c could be used to estimate P_N. Across a wide range of plant species, C_c is 85 ppm lower than C_i, and as a result, C_c-based V_{cmax} may be 60% lower than C_i-estimated V_{cmax} (Warren 2008). Values of g_m may oscillate during the day (Grassi *et al.* 2009) most likely in response to variations in irradiance, temperature or [CO_2] around the leaf (Flexas *et al*. 2008, Warren 2008). In Central Amazonia, g_s of saplings is higher around midday than early in the morning and the end of the afternoon (Magalhães 2010, Mendes and Marenco 2010). However, there is no information about how diurnal changes in g_s affect g_m of Amazonian trees.

In this paper, we hypothesized that g_m is influenced by diurnal variation of environmental factors in the same way they affect g_s. We also hypothesized that *M. paivae* and *M. guianensis* have similar g_m, and that variations in g_m are similar in both species under the ambient conditions of the forest understory. *M. guianensis* is a slow-growing, late-successional. It is highly valued by the timber industry because of the durability of its wood. Besides its uses for the production of railroad ties, fences, floors, and building bridges (Nebel 2001), *M. guianensis* has been also used as antimalarial, antihelmintic, and for cold treatment, and topically applied to relieve muscle pain and skin irritation (Marles *et al*. 1989, Cursino *et al*. 2012). *M. paivae* is considered a medicinal plant in the Amazon region. Trees of this species may reach up to 12 m height. An infusion of its leaves may be used as a tonic during human pregnancy in northwest Amazonia (Schultes and Raffauf 1993) or for diabetes treatment. Besides its medicinal properties, leaves can be used as a dye source. The objectives of this study were to determine the effect of time of day on photosynthetic parameters, and also to assess the effect of using either C_i or C_c on V_{cmax} in both *M. paivae* and *M. guianensis*.

Materials and methods

Study area and plant material: The study was conducted at the Tropical Forest Experiment Station (ZF-2 Reserve, 02º 36' 21"S, 60º 08' 11"W) of the National Institute for Research in the Amazon during the dry season of 2010. The area is a dense *terra firme* rainforest and the vegetation has high species richness, with about 300 species per ha (Oliveira and Mori 1999, Steege *et al.* 2003). In the area, the most abundant families are Lecythidaceae, Sapotaceae, and Burseraceae, and canopy trees, with hypostomatic leaves, may reach up to 30 m of height (Camargo and Marenco 2011). The soil type is an oxisol (yellow latossol in the Brazilian classification) with acid pH (3.5–4.0) and poor in nutrients (Mendes and Marenco 2010). In 2010, annual rainfall was 2,800 mm, and during the two-month study period, the mean temperature was 27°C, and the average soil moisture 65%, close to the soil saturation point (74%).

We used saplings of two shade-tolerant species, *M. paivae* O. Berg (Myrtaceae) and *M. guianensis* Aubl. (Olacaceae). Because of the high biodiversity (over 5,000 tree species across the Amazon region), trees from given species are distributed across a broad geographical area and a wide range of ages and sizes. After extensive search over an area of about 50 ha, four saplings (replications) of each species, 1 to 3 m tall and 8 to 19 mm in diameter (at 0.5 m from the ground) were finally selected for gas-exchange measurements. Saplings of these sizes were about 10 to 20 years old.

Gas-exchange and fluorescence measurements were conducted using a portable gas-exchange system (*Li-6400*, *Li-Cor*, Lincoln, NE, USA) coupled with a fluorometer (*Li-6400-40*, *Li-Cor*), and using a 2-cm^2-leaf chamber for collecting data. Data were collected between August and October 2010 (mild, dry season in Central Amazonia) on 2 fully expanded leaves per plant and 4 plants per species (1 plant per week). This season was chosen, because it is quite difficult to collect gas-exchange data in the rainy period. Photosynthetic rates were determined at different [CO_2] (P_N/C_i response curves) and under light saturation (photosynthetically active radiation – PAR of 500 μmol m^{-2} s^{-1}, this value was determined in preliminary experiments). P_{Nmax} was determined at light saturation and [CO_2] of 380 ppm. Leaf respiration in the light (R_L) was determined at low light intensity, as the intercept of the P_N/PAR regression line on the y axis (Villar *et al*. 1994).

After a stabilization period at [CO_2] of 380 ppm, light saturation, and ambient temperature (27°C), P_N/C_i response curves were generated varying [CO_2] in the leaf chamber (Long and Bernacchi 2003). We used [CO_2] of 380, 250, 100, 50, 380, 550, 1,000; 1,500; and 2,000 ppm. V_{cmax} was calculated according to Farquhar *et al.* (1980) using [CO_2] in the chloroplast ($V_{cmax\text{-}Cc}$). For comparison, C_i-based V_{cmax} values ($V_{cmax\text{-}Ci}$) were also obtained. V_{cmax} values were standardized to 25°C, using the equations described by Medlyn *et al.* (1999). Maximum Chl fluorescence of a dark-adapted leaf (F_m) was determined by applying a saturating light pulse of 6,000 μmol m^{-2} s^{-1} PAR, 0.9 s duration. The quantum yield of photosystem (PS) II (Φ_{PSII}) and the electron transport rate (J_F) were calculated according to Maxwell and Johnson (2000) as:

$$\Phi_{PSII} = [(F_m' - F_s)/F_m'] \quad (1)$$

$$J_F = 0.5\ (I_e \times \Phi_{PSII}) \quad (2)$$

where: F_s and F_m' indicate the steady-state (dynamic equilibrium) and maximum fluorescence of an illuminated leaf, respectively. I_e denotes PAR absorbed by the leaf, 0.5 is the fraction of quanta absorbed by PSII relative to PSI. As a fraction of electron flux through the electron transport chain is used in alternative reactions, which reduce O_2 (*e.g.* water-water cycle), J_F values were corrected as follows (Gilbert *et al.* 2012): $J_{F\text{-}cal}$ = m J_F; where: m = 4 (P_N + R_L)/(PAR × Φ_{PSII} × 0.425). In this equation, P_N and R_L were obtained under nonphotorespiratory conditions (1% O_2 and 99% N_2). C_c values were determined as follows (Epron *et al.* 1995):

$$C_i = C_c\ (S^*/S) \quad (3)$$

where S^* denotes the specificity factor of Rubisco calculated as the slope (coefficient) of the J_c/J_o against C_i/O regression line and S the Rubisco specificity *in vitro* (2,560 mol mol^{-1}, Harley *et al.* 1992). The parameters J_c and J_o represent the flow of electrons used for carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP), respectively. J_c and J_o were calculated as follows (Epron *et al.* 1995):

$$J_c = 1/3\ [J_{F\text{-}cal} + 8\ (P_N + R_L)] \quad (4)$$

$$J_o = 2/3\ [J_{F\text{-}cal} - 4\ (P_N + R_L)] \quad (5)$$

where $J_{F\text{-}cal}$ (*i.e.* J_F corrected) corresponds to the sum of J_c and J_o ($J_{F\text{-}cal}$ = J_c + J_o). It refers to the total flow of electrons used in the activity of Rubisco. Values of J_F were determined as described previously (Eq. 2). The C_i/O ratio denotes the relationship between C_i and the oxygen concentration in intercellular spaces (O_2, 210 mmol mol^{-1}). Values of g_m were determined as $g_m = P_N/(C_i - C_c)$. In order to compare with data obtained using the IRGA-fluorescence method, g_m was also calculated using the IRGA method (Sharkey *et al.* 2007). To assess the effect of time of day on g_m, g_s, P_{Nmax}, V_{cmax}, and $J_{F\text{-}cal}$, we collected data at three different times (08:00–09:30, 12:00–13:30, and 15:00–16:30 h), using the same leaves on each occasion.

Leaf morphology and ambient conditions: In order to assess the effect of leaf morphology on gas-exchange parameters, we measured fresh leaf thickness (L_T) on 2 leaves per plant and 4 plants per species using digital calipers (precision of 0.01 mm). L_T was determined on the middle portion of the leaf blade and at two different points per leaf avoiding major veins. Leaf area was determined in the same leaves used to assess L_T with an area meter (*Li-3000, Li-Cor*, USA). The leaves were oven-dried at 72ºC until they reached constant mass. Specific leaf area (SLA) was determined as the area to leaf mass ratio.

During the study period, PAR, air relative humidity (RH), air temperature (T_{air}), and rainfall data were collected. PAR, RH, and T_{air} data were recorded using specific sensors (*Humitter 50Y, Vaisala Oy*, Finland and *Li-190SA, Li-Cor*, NE, USA) connected to a data logger (*Li-1400*, *Li-Cor*, NE, USA). To characterize ambient conditions, one sensor was positioned a few meters apart from one of eight previously selected plants (at 1 m above the forest floor). It remained there for one week (when IRGA data from this plant were collected), then the same sensor was moved to another plant for the next seven days and so on up to the entire cycle was completed. Rainfall data were collected with a conventional rain gauge, positioned at the top of a 40-m-observation tower, 3.3 km away from the study area. During the study, we also collected five soil samples around each one of the selected plants (aggregated of 40 samples over the study period) to determine soil moisture (S_u). S_u was determined as $(M_u - M_s)/M_s$, where M_u and M_s represent the mass of wet and dry soil, respectively.

Statistics: To examine the differences between species and the effect of time of day, data were subjected to analysis of variance (*ANOVA*). As the same leaves were measured during the course of the day, data were analyzed using a repeated measures analysis of variance. *Tukey*'s post-hoc test ($p \leq 0.05$) was used to assess the effect of time of day on study parameters. The effect of L_T, SLA, understory PAR, T_{air}, and RH on g_s, g_m, V_{cmax}, and other gas exchange variables were examined by regression analysis. Statistical analyzes were carried out using the *SAEG 9.0* statistical packet of the Federal University of Viçosa, MG, Brazil.

Results

During the study period, weekly rainfall ranged between 10 and 45 mm and daily relative humidity ranged between 80 and 90%. At the forest understory, mean daily PAR was 14 μmol m^{-2} s^{-1} with sunflecks of varying duration and intensity. Daytime understory temperature oscillated between 23 and 28°C (Fig. 1).

Higher g_s values were found in the morning than in the afternoon [0.06 *vs.* 0.34 mol(H_2O) m^{-2} s^{-1}, $p \leq 0.05$, Fig. 2*A*]. However, there was no difference between species, nor the interaction between species and time of day was significant. P_{Nmax} and V_{cmax} values did not differ between species. Although V_{cmax} tended to remain constant during the day, P_{Nmax} was higher at midday and in the morning than in the afternoon. For these two parameters, the interaction between species and time of day was not significant; thus, an average of P_{Nmax} values across species was shown (Fig. 2*B*). $V_{cmax\text{-}Ci}$ followed the same trend observed for $V_{cmax\text{-}Cc}$ (*i.e.* there was no difference between species or time of day). However, V_{cmax} values calculated using C_c ($V_{cmax\text{-}Cc}$) were about 25% higher than those calculated using C_i (inset in Fig. 2*C*).

$J_{F\text{-}cal}$ was higher in the morning than in the afternoon (Fig. 2*D*), without any difference between species. The interaction between species and time of day was not also significant. This indicated that the diurnal effect on $J_{F\text{-}cal}$ was similar in both species. Values of g_m obtained with the IRGA-fluorescence method were higher in *M. guianensis* than in *M. paivae*, and higher at midday and in the morning than in the afternoon (Fig. 2*E*); but the interaction between species and time of day was not significant. It suggests that the diurnal effect on g_m was similar in both species. With regards to methods for determining g_m, the IRGA method yielded g_m values almost twice as high as those recorded with the IRGA-fluorescence method, but the IRGA method failed to detect either diurnal effect or differences between species (Fig. 2*F*). It indicated this method as less sensitive than the fluorescence method. There was no significant correlation between g_m and air temperature, and air relative humidity or understory light. For L_T and SLA, mean values were 0.17 mm (± 0.028 mm SD) and 15.4 $m^2\ kg^{-1}$ (± 2.0 $m^2\ kg^{-1}$ SD), respectively; without differences between species. Finally, L_T did not lead to significant variation in g_m, g_s, P_{Nmax}, V_{cmax}, or $J_{F\text{-}cal}$ (Table 1).

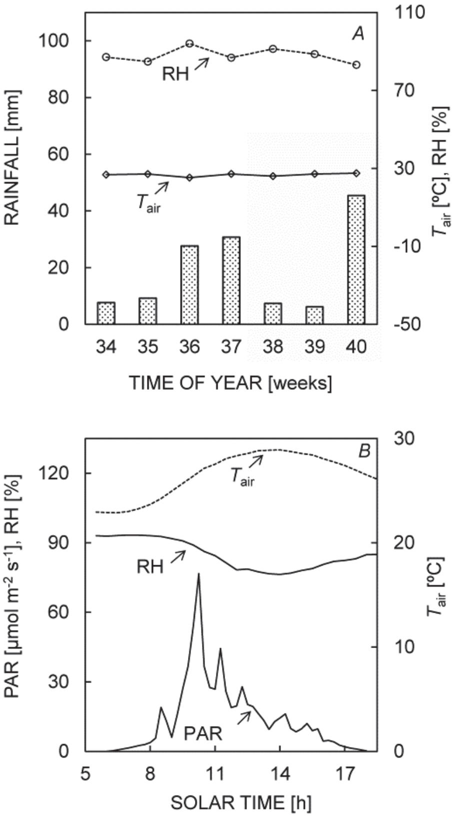

Fig 1. Weekly rainfall (*A*, *dotted bars*) and weekly mean temperature (T_{air}) and air relative humidity (RH). T_{air}, RH, and photosynthetically active radiation (PAR) as a function of time (*B*) during the study period, from August 22 to October 10 of 2010.

Discussion

Annual rainfall observed in 2010 is within the range previously reported for the same region (Dias 2009). Also, PAR values, air temperature, and relative humidity recorded at the forest understory concur with those previously reported by Mendes (2012) during the mild dry period of Central Amazonia. The mean g_s value observed in the morning is similar to that reported by Magalhães (2010) for tree species in the juvenile stage in the Central Amazon. Decline of g_s during the day confirms results previously reported by others (Kaiser and Kappen 2000, Mendes and Marenco 2010).

The lower values of g_s at the end of the day could not be attributed to low availability of water in the soil, because soil moisture (65%) was close to soil field capacity throughout the study period due to rainfall events, which occurred during the campaign for data collection (Fig. 1*A*). High soil moisture ruled out the possibility of plants having experienced water stress throughout the day. As ambient conditions were kept nearly constant within the leaf chamber (*e.g.* saturated light intensity and 380 ppm CO_2), the drop in g_s values in the afternoon does not seem to be related to external factors. It has been suggested that a biological clock may modulate stomatal opening and closing in some tree species (Doughty *et al.* 2006, Mendes 2012), perhaps involving circadian rhythms. Entraining stomata opening to a biological clock increases light-use efficiency during the day and prevents unnecessary loss of water by transpiration at night (Dodd *et al.* 2005).

The lower values of P_{Nmax} were found at the end of the day, when the irradiance was lower in the forest understory, which is consistent with P_{Nmax} values observed in young Amazonian tree species (Carswell *et al.* 2000). V_{cmax} values observed in this study are within the range reported for other tropical forest species, between 14 and 28 µmol(CO_2) $m^{-2}\ s^{-1}$ (Magalhães 2010). In comparison with $V_{cmax\text{-}Cc}$, the lower $V_{cmax\text{-}Ci}$ values confirm that the use of C_i instead of C_c leads to underestimation of real V_{cmax} values (Manter and Kerrigan 2004). In fact, V_{cmax} can be underestimated by 50% (and up to 100%, for plants under water stress), when C_i instead of C_c is used in V_{cmax} calculation (Flexas *et al.* 2007, Warren 2008).

The low P_{Nmax} values observed in the afternoon can

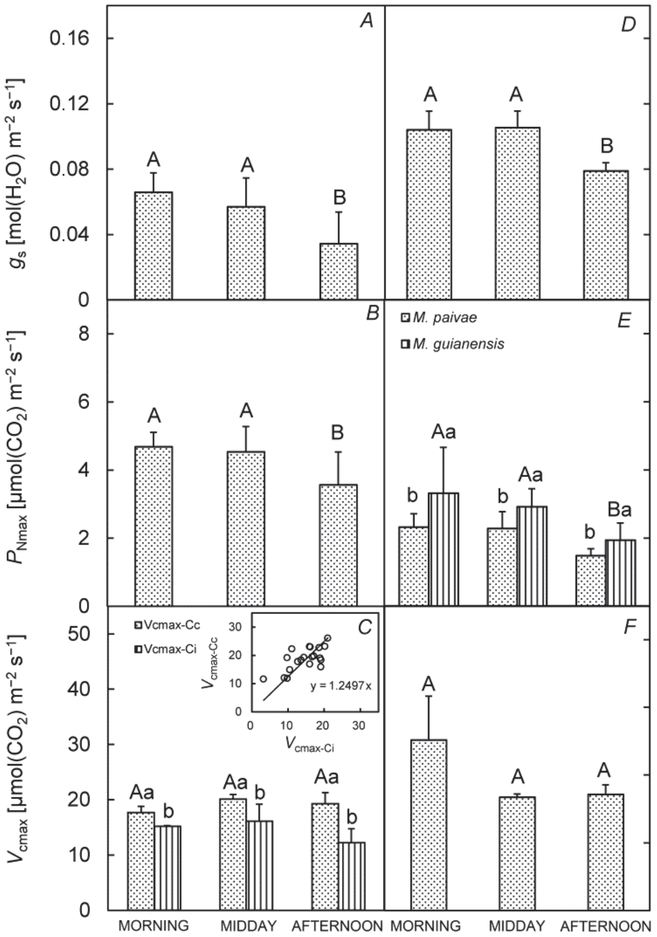

Fig. 2. Effect of time of day in stomatal conductance (g_s, *A*), light-saturated net photosynthetic rate (P_{Nmax}, *B*), the maximum carboxylation velocity of Rubisco (V_{cmax}, *C*), electron transport rate ($J_{F\text{-}cal}$, *D*), mesophyll conductance (g_m) determined by either the gas exchange–chlorophyll fluorescence method (*E*) or the gas-exchange method alone (*F*) in *M. paivae* and *M. guianensis*. On panels *A*, *B*, *D*, and *F* each bar represents the mean of 2 species, 4 plants per species, and 2 leaves per plant. On panel *E* each bar represents the mean of 4 plants per species and 2 leaves per plant. Bars (± SD) with *the same letters* [*uppercase* for time of day and *lowercase* for V_{cmax} (*C*) or species (*E*)] do not differ at 5% probability. *The inset* on panel *C* shows the relationship between $V_{cmax\text{-}Cc}$ and $V_{cmax\text{-}Ci}$.

Table 1. Correlation coefficients (r, n = 8) between stomatal conductance (g_s), mesophyll conductance (g_m), light-saturated net photosynthetic rate (P_{Nmax}), maximum carboxylation velocity of Rubisco (V_{cmax}), and electron transport rate ($J_{F\text{-}cal}$) and specific leaf area (SLA) and leaf thickness. SLA values ranged between 11.15 and 17.77 m^2 kg^{-1} and leaf thickness (L_T) between 0.14 and 0.23 mm. ns – not significant at 5% probability.

Variables	SLA	L_T
g_s	-0.55^{ns}	0.06^{ns}
g_m	0.2^{ns}	0.28^{ns}
P_{Nmax}	0.14^{ns}	0.41^{ns}
V_{cmax}	0.41^{ns}	0.04^{ns}
$J_{F\text{-}cal}$	0.1^{ns}	0.31^{ns}

be attributed to decline in g_s and g_m at the end of the day (Fig. 2*A*,*E*), which is in agreement with the results reported by others (Evans and Loreto 2000, Flexas *et al.* 2007). Because V_{cmax} did not experience diurnal changes (Fig. 2*C*), reduction of P_{Nmax} in the afternoon seems to be related to the combined effect of diffusive and photochemical factors, as both g_s and $J_{F\text{-}cal}$ were lower in the afternoon. Some studies have shown that Rubisco activity changes during the day, with low activity around midday (Parry *et al.* 1993, Hrstka *et al.* 2007). This variation did not occur in this study. Thus, the absence of a diurnal effect on V_{cmax} suggests that factors affecting Rubisco activity, such as enzyme activation, experienced little or no change during the day, which ultimately resulted in stable V_{cmax} values. The afternoon decline in $J_{F\text{-}cal}$ observed in this study is similar to that recorded in forest species by Ishida *et al.* (1999), who observed lower electron transport rates after 14:00 h. Reduction in $J_{F\text{-}cal}$ in the afternoon might, at least partially, explain the drop in P_{Nmax} at the end of the day. A close relationship between P_{Nmax} and $J_{F\text{-}cal}$ has been reported in several studies. For example, similar decline in both $J_{F\text{-}cal}$ and P_{Nmax} at sunset has been reported in other plant species (Flexas *et al.* 1999, Medrano *et al.* 2002). In the absence of g_m data for Amazonian tree species, g_m for other tree species were used for comparison. Our g_m values were lower than those reported for evergreen species [about 0.1 mol(CO$_2$) m^{-2} s^{-1}, Flexas *et al.* 2012], *Eucalyptus globulus* or orange (Warren 2004, Magalhães-Filho *et al.* 2009). Both g_m and g_s had similar values (about 0.05 mol m^{-2} s^{-1}), which is in agreement with Flexas *et al.* (2012), who reported that these two parameters may limit photosynthesis in the same proportion. Higher g_m values in *M. guianensis* negated our initial hypothesis. It suggests that even when ambient conditions are rather similar in the forest understory, interspecific variation in leaf traits other than leaf thickness or specific leaf mass may lead to varying g_m (Flexas *et al.* 2008).

The variation of g_m throughout the day followed the same pattern as observed in g_s. That is, higher g_s values were recorded in the morning. Values of g_m did not correlate with atmospheric variables (T_{air}, PAR, RH), which suggests that environmental changes in the forest understory are not large enough to cause variation in g_m. This led us to conclude that endogenous factors might somehow contribute to diurnal variation in g_m.

In some plants, photosynthesis responds to the activity of a biological clock (McClung 2000), which opens up possibility for g_m to be also influenced by endogenous factors. It has also been suggested that aquaporins are involved in CO_2 transport to the chloroplast, and thus, they can affect g_m (Sarda *et al.* 1997, Flexas *et al.* 2012). Diurnal changes in g_m can be associated to variation in aquaporin gene expression as reported by Lopes *et al.* (2003).

The difference in g_m values obtained by the two methods (IRGA *vs.* IRGA-fluorometer) is contrary to what should be expected on the basis of the previous work by Flexas *et al.* (2007). Our results, however, concur with those reported by Pons *et al.* (2009), who observed only good correlation between both methods at some [CO_2]. SLA and L_T values reported in this study are in agreement with previous reports (Magalhães 2010, Mendes and Marenco 2010). Although some studies have reported a significant correlation between g_m and L_T (Syvertsen *et al.* 1995, Flexas *et al.* 2012), we failed to detect such an effect, perhaps because changes in L_T were not large enough to affect g_m or because other anatomical parameters (*e.g.* path length for CO_2, pathway tortuosity for CO_2 diffusion, and cell wall porosity) somehow offset the variation in L_T. However, our results agree with those of Evans *et al.* (1994), who found no correlation between L_T and g_m. The lack of correlation between V_{cmax} and L_T or V_{cmax} and SLA might be related to absence of correlation between g_m and g_s and L_T. This suggests that under the ambient conditions of the forest understory, plant variation in leaf structure either does not influence CO_2 diffusion to the chloroplasts or that the changes in L_T are too small to affect V_{cmax} in studied species.

Decline in photosynthetic rates at the end of the afternoon seems to be associated with variations in g_s and g_m rather than changes in biochemical parameters. It seems possible that diurnal variations in endogenous factors somehow lead to changes in g_m, specific factors involved remain to be elucidated. Even when *M. guianensis* and *M. paivae* share rather similar environmental conditions at the forest floor, they have different g_m values. More studies are required for a better understanding factors that affect photosynthetic parameters of Amazonian tree species. Finally, whenever possible, Chl fluorescence should be used as a complement to gas exchange in order to obtain more robust g_m values. C_c instead of C_i should be used in V_{cmax} calculation.

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