

Photosynthetic parameters of two invasive tree species of the Brazilian Pantanal in response to seasonal flooding

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Abstract

The Pantanal is the largest wetland in the world with extremely high plant and animal diversity, but large areas have been invaded by *Vochysia divergens* Pohl (Vochysiaceae), a tree that is native to the Amazon Basin, and *Curatella americana* L. (Dilleniaceae), a tree that is native to the Brazilian savanna (cerrado). *V. divergens* is reportedly flood-adapted, thus its ability to invade the Pantanal may not be surprising, but the invasion of *C. americana* is counterintuitive, because this species is adapted to the well-drained soils of the cerrado. Thus, we were interested in comparing the photosynthetic capacity, in terms of CO₂ conductance, carboxylation, and electron transport of these species over a seasonal flooding cycle. Given that *V. divergens* is reportedly flood-adapted, we predicted that this species would have a higher photosynthetic capacity than *C. americana*, especially under flooding. To test this hypothesis we measured the photosynthetic CO₂ response (P_N/C_c) of *V. divergens* and *C. americana* within 1 year to determine, if photosynthetic capacity varied systematically over time and between species. Contrary to our hypothesis, *V. divergens* did not always have a higher photosynthetic capacity than *C. americana*. Rather, species differences were influenced by temporal variations in flooding and the leaf age. Leaf CO₂ assimilation and photosynthetic capacity of both species were lower during the flood period, but the differences were not statistically significant. The physiological performance of both species was strongly related to leaf N and P concentrations, but P limitation appeared to be more important than N limitation for these species and ecosystem. Photosynthetic capacity was higher and more stable for *V. divergens*, but such an advantage did not result in a statistically significant increase in P_N . Our results suggest that both species are tolerant to flooding even though they are adapted to very different hydrological conditions. Such physiological plasticity, especially for *C. americana*, might be a key feature for the ability to survive and persist in the seasonally flooded Pantanal.

Additional key words: cerrado; *Curatella americana* L.; invasive species; neotropical *Vochysia divergens* Pohl; Pantanal; wetlands.

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Abbreviations: C_c – molar concentration of CO₂ in the chloroplast; C_i – molar concentration of CO₂ in the intercellular air space; ET – evapotranspiration; g_m – mesophyll conductance; g_s – stomatal conductance; J_{max} – light-saturated rate of electron transport; L – leaf age; N – concentration of nitrogen; P – concentration of phosphorus; P_N – net photosynthesis; P_N/C_c – photosynthetic response curves to variations in concentration of CO₂ in the chloroplast; PPFD – photosynthetic photon flux density; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; S – species; SLA – specific leaf area; T – time; TPU – triose phosphate utilization; T_l – leaf temperature; V_{cmax} – maximum rate of Rubisco activity; VPD – atmospheric vapor pressure deficit; YL – young leaves; OL – old leaves; Γ – CO₂ compensation point; Γ^* – CO₂ compensation point in the absence of mitochondrial respiration.

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Introduction

The Pantanal is the largest wetland in the world with an area of 150,000 km² that extends over three countries (Brazil, Paraguay, and Bolivia), most of which (71%) is located in the Brazilian states of Mato Grosso and Mato Grosso do Sul (Haase 1999). Most of the wetland is subjected to seasonal flooding that results from local precipitation patterns, run off to large river systems, and impeded drainage of rain water through soil (Nunes da Cunha and Junk 2001, Arieira and Nunes da Cunha 2006). The Pantanal has extremely high plant species diversity, with approximately 144 families and over 1,000 plant species (Junk and Nunes da Cunha 2005); however, this diversity is increasingly vulnerable due to land use practices, alterations in hydrology and climate, and invasive species (Junk *et al.* 2006).

In terms of invasive species, the spread of *Vochysia divergens* Pohl (commonly known as cambará) in the Pantanal has been well-documented over the last four decades (Zeilhofer and Schessl 1999, Nunes da Cunha and Junk 2001, Junk and Nunes da Cunha 2005, Arieira and Nunes da Cunha 2006, Junk *et al.* 2006). Cambará is a flood-adapted, pioneer tree species native to the Amazon (Pott and Pott 1994) that has invaded extensive areas of the Pantanal, forming monocultures popularly known as cambarazais. Although the mechanisms for the spread of cambará are poorly understood, high rainfall causes prolonged flooding, which allows *V. divergens* seedling recruitment before the onset of the dry season, when soil moisture may become limiting (Nunes da Cunha and Junk 2004).

The invasion of *Curatella americana* L. (commonly known as lixeira) to the Brazilian Pantanal has also been recently observed (Santos *et al.* 2006), and contrary to the flood-adapted *V. divergens*, *C. americana* is native to the Brazilian savanna (cerrado) (Medina and Francisco 1994, Lorenzi 2002), which is an upland ecosystem consisting of well-drained soils, where water-limitation is typical during the dry season and flooding is uncommon (Eiten 1972, Furley and Ratter 1988). The mechanism of this invasion is poorly known; however, lixeira apparently expands into the Pantanal from the surrounding cerrado during times of low water and becomes established before flooding ensues during the wet season (Santos *et al.* 2006).

Many factors may contribute to the invasion success,

including high relative growth rate, seed production and germination, seedling recruitment, carbon allocation, life history characteristics of species, and susceptibility to disease and/or herbivores (Rejmánek and Richardson 1996, Mack *et al.* 2000, Grotkopp and Rejmánek 2007). Especially in the Pantanal, the ability to tolerate flooding is another, highly relevant factor for the invasion success of plant species. Floods cause changes in the chemical, physical, and biological soil properties (Pezeshki and Delaune 1998, Parolin *et al.* 2006, Kramer *et al.* 2008), affecting negatively the physiological functioning of many tree species. However, many species have developed a wide range of adaptive strategies that make them able to tolerate periodic flooding (Pezeshki *et al.* 1996, Iwanaga and Yamamoto 2008), allowing them to maintain a high photosynthetic activity during most of the year. This tolerance may allow these species to dominate over the natural competitors that may be more efficient under nonflooding conditions, but unable to compete when flooded (Parolin *et al.* 2010a).

Understanding a problem how physiological performance is affected by environmental variation can help to elucidate possible mechanisms for a survival in novel and potentially unfavorable environments (Sharkey *et al.* 2007, McDowell *et al.* 2008). For this purpose, measurements of the biophysical and biochemical mechanisms of leaf gas exchange have been successfully used to quantify the environmental and genetic influences on plant productivity (Sharkey *et al.* 2007). While there is a debate on the link between photosynthesis and plant growth (*e.g.* Rhodenbaugh and Pallardy 1993, Sinclair *et al.* 2004), there is considerable evidence that variations in photosynthetic capacity can directly affect a biomass production (Long *et al.* 2006, von Caemmerer and Evans 2010). Thus, the aim of this study was to evaluate the monthly variations in photosynthetic parameters of two invasive species in response to changes in the leaf age, morphology, and nutrient content (N and P) in the seasonally flooded Brazilian Pantanal. Whereas many species of Amazonian floodplain trees show their greatest photosynthetic activity during the flood (Piedade *et al.* 2010), we hypothesized that *V. divergens*, known for being adapted to flooding, should have greater photosynthetic capacity than *C. americana*, especially during this period.

Materials and methods

Study site: The study was conducted between May 2009 and April 2010 in the northern Pantanal (16°39'50"S, 56°47'50"W) about 160 km SSW of Cuiabá, Mato Grosso, and 60 km SE of Poconé, Mato Grosso, Brazil. The study site is located 111 m above the sea level in a protected nature reserve, known as the Private Reserve of

Natural Heritage (RPPN) of the Social Service of Commerce (SESC)-Pantanal. The site is in a transition zone between a monodominant cambará forest and a natural savanna, where both *V. divergens* and *C. americana* co-occur. Besides *V. divergens* and *C. americana*, the most common vegetation consists of a grass,

Gymnopogon spicatus (Spreng.) Kuntze (Poaceae), and perennial herb, *Mimosa pellita* H. et B. (*Mimosa pellita* Kunth ex Willd.) (Vourlitis *et al.* 2011). The soil is classified as Solonetz Gleyic (Zeilhofer 2006) with sandy loam texture, low organic matter content [10–15 g kg⁻¹ (dry soil)], and pH of 4.7 (Vourlitis *et al.* 2011).

Annual rainfall of the region is approximately 1,400 mm with a dry season extending from May to September, when there may be no measurable rainfall (Nunes da Cunha and Junk 2001). The topography of the floodplain is virtually flat, causing extensive flooding during the wet season (Nunes da Cunha and Junk 2001). Surface flooding occurs typically between December and May, with peak water levels ranging between 1–2 m in depth (Nunes da Cunha and Junk 2004).

Species description: *C. americana* is a tree species native to the Brazilian cerrado that reaches 6–10 m in height (Medina and Francisco 1994, Lorenzi 2002). It has semideciduous phenology, with leathery leaves that have a life-span of approximately 10 months (A. Dalmolin, unpublished results). New leaves are produced during *ca.* 1 month period during the dry season, typically in July, and between July and October, two leaf cohorts are present at the same time, one about 7–8 months old, which developed during the previous season (hereafter referred to as “old” leaves, OL), and another one, which developed during the current season (hereafter referred to as “young” leaves, YL).

V. divergens is an evergreen tree native to the Amazon Basin that can reach a height of 25–28 m (Pott and Pott 1994). The leaves are glossy and dark green, having a life span of 8–9 months (A. Dalmolin, unpublished results), they are produced regularly throughout the year, but with a break in the flooding period (*ca.* December–March). Similarly to *C. americana*, it is possible to identify two cohorts of leaves during the dry season, one, which developed during the previous year (OL), and another, which developed during the current year (YL).

Leaf gas exchange: We randomly selected five young plants of both *C. americana* and *V. divergens*, 3–5 m high, looking healthy, and exposed to the sun; they were distributed in an area of approximately 0.5 ha in the study site, subjected to the same flooding. When possible, we measured rates of gas exchange on both YL and OL of both species, because their physiological performance is known to be affected by the leaf ontogeny (Kitajima *et al.* 2002). YL were defined as the first, fully-expanded, dark-green leaves that were closest to the stem apex, while OL were located at least 4 leaf pairs below the stem apex and they were tougher in a texture with a mixture of dark-green, yellow, and brown patches.

The photosynthetic response to the partial pressure of CO₂ at the sites of carboxylation (P_N/C_c) was measured monthly for each species using a portable photosynthesis

system (*LI-6400*, *LI-COR*, Lincoln, NE, USA). Measurements were made during the morning on sunny days, avoiding the midday hours, when there was a reduction in photosynthesis. Leaves were placed in the *LI-6400* chamber, which was adjusted to provide a constant photosynthetic photon flux density (PPFD) of 1,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ to ensure light saturation for photosynthesis, 28°C block temperature, and relative humidity of 60%. After 15 min of acclimation to these conditions, the *LI-6400* was adjusted to vary the chamber CO₂ concentration in the following order: 400, 300, 250, 200, 150, 100, 50, 400, 400, 450, 500, 600, 700, 800; 1,000; and 1,200 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$. The assimilation was recorded at each change in CO₂ concentration and only after the coefficient of variation was < 0.3%.

The parameters of the curve P_N/C_c were then calculated using the well-known Farquhar *et al.* (1980) model, which summarizes the dependence of the net carbon assimilation rate (P_N [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$]) on the partial pressure of CO₂ at the sites of carboxylation (C_c [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$]). P_N/C_c curves were used instead of P_N/C_i curves, which are more often reported in the literature, because they rely on mesophyll conductance (g_m), thus avoiding the underestimation of $V_{c\text{max}}$ (Epron *et al.* 1995, Manter and Kerrigan 2004).

P_N/C_c curves can be modeled as the minimum of three limiting rates:

$$P_N = \min \{P_c, P_j, P_p\} - R_d \quad (1)$$

where P_N is the net CO₂ assimilation rate [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$]; $\min\{\}$ denotes “the minimum of”; P_c , P_j , and P_p are the net CO₂ assimilation limited by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), ribulose-1,5-bisphosphate (RuBP) and triose-phosphate utilization (TPU), respectively [$\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$]; R_d is the rate of mitochondrial respiration [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$].

Rubisco-limited photosynthesis is expressed as:

$$P_c = V_{c\text{max}} (C_c - \Gamma^*) / C_c + K_c (1 + O/K_o) \quad (2)$$

where $V_{c\text{max}}$ is the maximum rate of Rubisco activity [$\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$]; C_c is the molar concentration of CO₂ in the chloroplast [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$]; O is the molar concentration of O₂ at Rubisco [$\mu\text{mol}(\text{O}_2) \text{mol}^{-1}$]; Γ^* is the CO₂ compensation point in the absence of mitochondrial respiration [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$]; K_c and K_o are the Michaelis-Menten coefficients of Rubisco activity for CO₂ and O₂, respectively [$\mu\text{mol} \text{mol}^{-1}$]. The rate of photosynthesis limited by RuBP regeneration is expressed as:

$$P_j = J (C_c - \Gamma^*) / 4 C_c + 8 \Gamma^* \quad (3)$$

In Eq. 3, the potential electron transport rate (J) is related to J_{max} through an empirical relationship (Farquhar and Wong 1984):

$$\theta J^2 - (\alpha \text{PPFD} + J_{\max})J + \alpha \text{PPFD} J_{\max} = 0 \quad (4),$$

where J is the potential rate [$\mu\text{mol m}^{-2} \text{s}^{-1}$] of electron transport that is dependent upon photosynthetic photon flux density (PPFD); θ is the curvature of the light response curve, and α is the quantum yield of electron transport. The value of α was fixed at 0.3 [$\text{mol}(\text{electron}) \text{mol}(\text{photon})^{-1}$], based on an average C_3 photosynthetic quantum yield of 0.093 and a leaf absorbance of 0.8 (Long *et al.* 1993). The value of θ was taken to be 0.90. J_{\max} is the light-saturated rate of electron transport [$\mu\text{mol m}^{-2} \text{s}^{-1}$]. The rate of photosynthesis limited by TPU is expressed as:

$$P_p = 3 \text{ TPU} \quad (5),$$

where TPU is the rate of phosphate release in triose-phosphate utilization [$\mu\text{mol m}^{-2} \text{s}^{-1}$].

C_c is related to C_i , P_N , and g_m , which is the mesophyll conductance [$\mu\text{mol m}^{-2} \text{s}^{-1} \text{mol}^{-1}$]. P_N/C_i curve data can be used to calculate g_m through Eqs. 2, 3, and the following equation:

$$C_c = C_i - P_N/g_m \quad (6),$$

where C_i is the molar concentration of CO_2 in the intercellular air space [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$].

The temperature response of parameters was estimated following Harley *et al.* (1992):

$$\text{Parameter} = e^{\left(c - \frac{\Delta H_a}{RT_1}\right)} \quad (7),$$

or

$$\text{Parameter} = \frac{e^{\left(c - \frac{\Delta H_a}{RT_1}\right)}}{1} + e^{\left(\Delta S T_1 - \frac{\Delta H_d}{RT_1}\right)} \quad (8),$$

where c is a scaling constant, ΔH_a is enthalpy of activation [KJ mol^{-1}], ΔH_d is enthalpy of deactivation [KJ mol^{-1}], ΔS is entropy, R is gas constant [$8.314 \text{ J mol}^{-1} \text{ K}^{-1}$], and T_1 is a leaf temperature [273.15 K].

To analyze P_N/C_c curve, the constants and functions of temperature had their parameters corrected to 25°C using coefficients previously reported by Harley *et al.* (1992) and Sharkey *et al.* (2007).

Results

Climatology during the measurement period: Total rainfall was approximately 1,550 mm during the May 2009–April 2010 measurement period, which was slightly above the long-term average of 1,400 mm for the region. Between May and November 2009, rainfall was insufficient to offset the potential evapotranspiration (ET), leading to a decline in a surface water level (Fig. 1A). During this time, the average monthly air temperature increased from a minimum of 22.4°C in June to

Leaf physical and chemical determinations: After the P_N/C_c curve measurements, leaves were removed and their fresh leaf area measured with a portable leaf area meter (*CI-202*, *CID Inc.*, Camas, WA, USA). Leaves were then dried at 70°C and weighed using an analytical balance (*AG 200*, *GEHAKA*, São Paulo, SP, Brazil). Specific leaf area (SLA) was calculated by dividing the one-sided fresh leaf area [cm^2] by the dry mass (DM) of the leaf [g]. Dried material was then ground, and N leaf concentration was determined using digestion method and micro-Kjedahl digestion (Golterman *et al.* 1978); P content was determined by colorimetric analysis using vitamin C method (Braga and Defelipo 1974).

Statistical analysis: Multivariate analysis of variance (*MANOVA*) was used to determine whether there were statistical differences in the dependent variables: P_N , R_d , Γ , g_s , V_{cmax} , J_{max} , and TPU per unit area as a function of species (S), leaf age (L), and time (T). For these analyses, P_N and g_s were measured at a CO_2 concentration of $400 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$, which is the closest to the ambient concentration, PPFD of $1,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, 28°C block temperature, and a relative humidity of 60% as described above. In the case of a significant interaction between the independent variables, a one-way analysis of variance was used to determine whether there were differences between S, L, or T.

P_N/C_c curve analysis was conducted using a spreadsheet developed by Su *et al.* (2009), written for *Matlab* (*V7.3*, *Matworks Inc.*, Natick, MA, USA). Following Su *et al.* (2009), the genetic algorithm (GA) was used to estimate the parameters of V_{cmax} , J_{max} , TPU, R_d , and g_m , which were randomly adjusted photosynthetic parameters to provide the best fit (based on the minimum mean square error) of the entire P_N/C_c curve. This approach minimizes subjective errors that may arise, when estimates of V_{cmax} , J_{max} , and TPU are individually fitted to estimate each parameter.

Stepwise linear regression was used to analyze the dependence of mass-based P_N , V_{cmax} , and J_{max} on specific leaf area (SLA) and leaf nutrient (N and P) concentrations. Data were grouped, when there was no significant difference in the slope or intercept between leaf age or species.

approximately 27°C by October (Fig. 1B). Similarly, the atmospheric vapor pressure deficit (VPD) increased from 0.75 kPa at the end of the wet season (May–June) to a peak of 1.35 kPa by September (Fig. 1B). For most of the 2009 dry season, there was no standing water above the soil surface and the soil water content in the upper 10 cm of the soil layer was $< 10\%$ by mass (Vourlitis *et al.* 2011). The rainy season began in October 2009, and by December, rainfall exceeded ET, but flooding had not

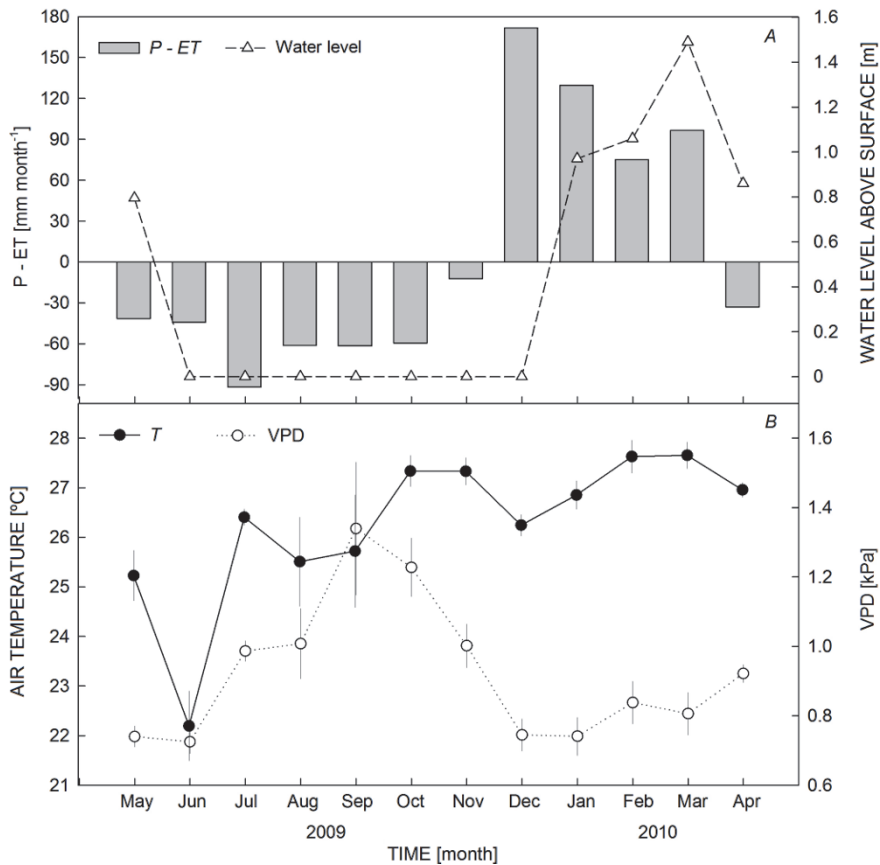


Fig. 1. (A) Average monthly precipitation – potential evapotranspiration (P-ET; shaded bars) and water level depth above the soil surface (open triangles, dashed lines) and (B) mean (\pm SD, $n = 30$) monthly air temperatures (solid circles, solid lines) and the atmospheric vapor pressure deficits (open circles, dotted lines) for the study site during the May 2009–April 2010 study period. Micrometeorological data were obtained from a micrometeorological tower installed 500 m from the study site (Sanchez *et al.* 2011).

begun yet. Flooding ensued by January 2010 and the surface water level was ≥ 1 m deep until May (Fig. 1A). The wet season air temperature was more consistent than during the dry season and ranged between 26.5–27.5°C, while VPD declined as soon as rainfall ensued in October and it was between 0.78–0.90 kPa, when the research site was flooded (Fig. 1B).

Variations in the leaf structure: *Vochysia divergens* had a cohort of the YL and OL from May–October 2009 and again in April 2010, while *Curatella americana* began producing new leaves in July and continued to produce the leaves till April 2010 (Fig. 2). By November, *C. americana* had shed most of the leaves produced during the previous year, but by April 2010, the leaves produced in the previous July aged enough to be considered “old” (see the description in Materials and methods).

New leaves of both species had a significantly higher specific leaf area (SLA), and both species exhibited significant temporal variations in SLA over time (Fig. 2). In general, both species had the highest SLA at the beginning of the wet season in October. In *V. divergens*, the SLA of the OL was more consistent (ca. 60–100 cm² g⁻¹) than that of the YL, which had a higher, but more temporally variable SLA (Fig. 2). In *C. americana*, the SLA for both YL and OL had the same temporal pattern as *V. divergens*, but the dynamic range in SLA was slightly higher (Fig. 2).

Photosynthesis vs. CO₂ response (P_N/C_c) curves: The P_N/C_c response was well described by Farquhar *et al.* (1980) model for both flood and dry season periods (Fig. 3) and for the YL and the OL (Fig. 4). Based on the sums of square errors (SSEs), the model appeared to perform better during flooding conditions and with the YL. In general, *V. divergens* did not exhibit large differences in the shape of the P_N/C_c curve over seasonal time scales, while *C. americana* had a lower P_N saturation level during the dry than the flood season (Fig. 3). In contrast, both species had higher rates of CO₂-saturated P_N in the OL (Fig. 4). Regardless of surface hydrology and the leaf age, P_N for *V. divergens* was primarily limited by Rubisco activity at a $C_c \leq 400$ $\mu\text{mol}(\text{CO}_2)$ mol⁻¹, while *C. americana* was primarily limited by Rubisco activity at a $C_c \leq 350$ $\mu\text{mol}(\text{CO}_2)$ mol⁻¹ (Fig. 3). The limitation by RuBP regeneration for both species was the most pronounced, when C_c was between 350–500 $\mu\text{mol}(\text{CO}_2)$ mol⁻¹, while the limitations by TPU were the most important at a $C_c > 500$ $\mu\text{mol}(\text{CO}_2)$ mol⁻¹.

Variations in leaf gas exchange and N and P concentration: MANOVA results revealed a significant effect of species, leaf age, and time (months) on the gas exchange variables and significant species vs. leaf age and species vs. time interactions (Table 1). Both species showed high rates of photosynthesis throughout the study period, with values always above 6.2 $\mu\text{mol}(\text{CO}_2)$ m⁻² s⁻¹ and reaching

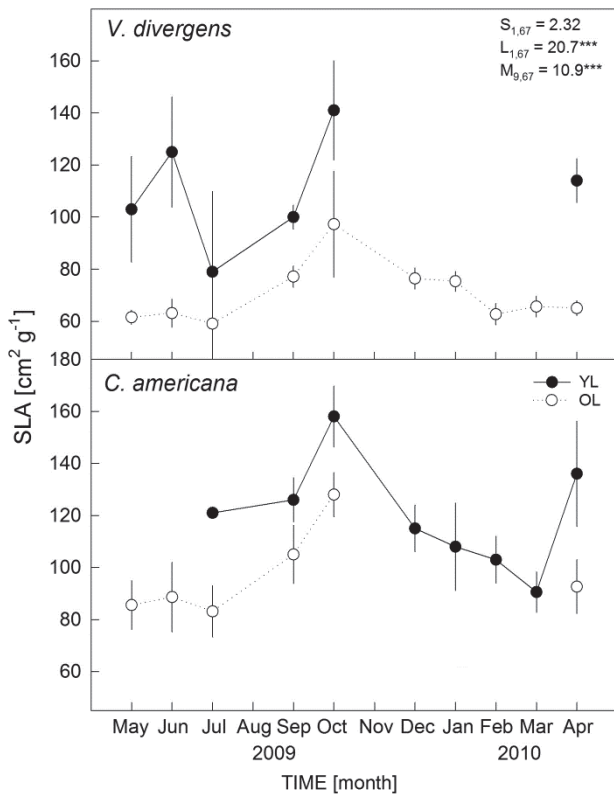


Fig. 2. Specific leaf area for young (YL, solid circles, solid lines) and old (OL, open circles, dotted lines) leaves of *V. divergens* (top panel) and *C. americana* (bottom panel) during the May 2009–April 2010 study period. The results of a 3-way ANOVA (F -statistics and degrees of freedom) are also shown for the main effect of species (S), leaf age (L) and season (T). Mean \pm SE ($n = 5$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

as high as $13.3 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 5). However, the leaf age was an important determinant for P_N ($F_{1,67} = 6.88$; $p < 0.01$), with the OL exhibiting higher rates of P_N than the YL, especially for *C. americana* (Fig. 5).

Stomatal conductance (g_s) varied significantly over time, but not as a function of species and the leaf age (Fig. 5). This variation appeared to be more pronounced for *V. divergens*, but it was not enough to differ significantly from *C. americana*. Rates of g_s were on the average lower for both species during the dry season and the dry-wet season transition (September–October), but interestingly, the old *V. divergens* leaves exhibited the highest g_s in June–July 2009 (Fig. 5), when air temperature and VPD were relatively low (Fig. 1B).

Values for the P_N/C_c curve parameters, V_{cmax} , J_{max} , and TPU were significantly higher in the OL, and for V_{cmax} and J_{max} significantly higher during the flood season (Fig. 6). These differences seemed to be the most evident for *C. americana*, accounting in part for the significant species vs. leaf age and species vs. time interactions observed with MANOVA (Table 1). For example, V_{cmax} for *C. americana* approximately doubled for the OL between the dry to flood seasons, while the increase in V_{cmax} for the YL from the dry to flood seasons was slightly smaller (Fig. 6). Similar trends were observed for J_{max} , but for TPU, the largest differences were observed for the leaf age (Fig. 6). For *V. divergens*, seasonal trends in V_{cmax} , J_{max} , and TPU were more variable, but all three parameters exhibited a transient decline for the OL during the peak of the dry season in July and a more sustained decline for the YL during the dry-wet season transition in September–October (Fig. 6).

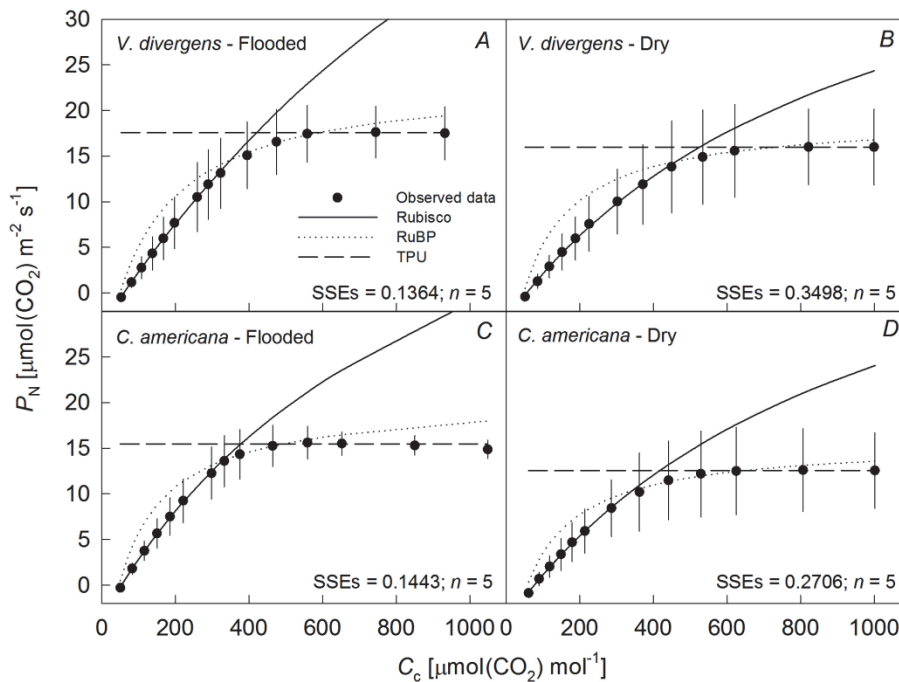


Fig. 3. Leaf photosynthesis response (P_N) versus molar concentration of CO_2 in chloroplast (C_c) for leaves of *V. divergens* (A and B) and *C. americana* (C and D) during flooding (A and C) and dry (B and D) periods. The Farquhar *et al.* (1980) model functions for Rubisco (solid line), RuBP (dotted line), TPU (dashed line) limitations on P_N , and the sum of squared error (SSEs) of the model and the number of data sampled per season (n) are also shown. Mean \pm 95% confidence interval.

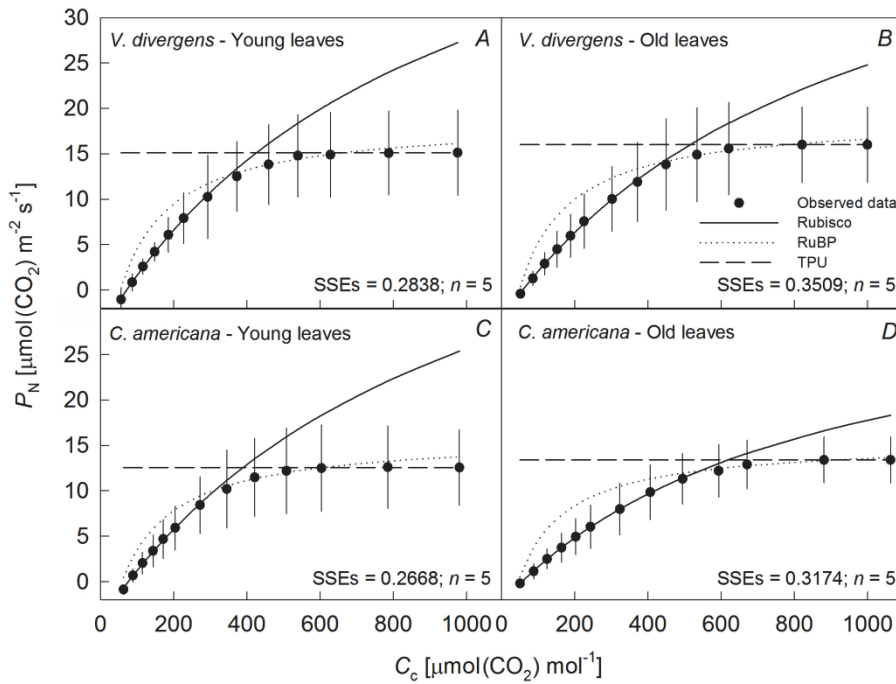


Fig. 4. Leaf photosynthesis response (P_N) versus molar concentration of CO_2 in chloroplast (C_c) for young (A and C) and old (B and D) leaves of *V. divergens* (A and B) and *C. americana* (C and D). The Farquhar *et al.* (1980) model functions for Rubisco (solid line), RuBP (dotted line), TPU (dashed line) limitations on P_N , and the sum of squared error (SSEs) of the model and the number of data sampled per season (n) are also shown. Mean \pm 95% confidence interval.

Table 1. Multivariate analysis of variance (MANOVA) results for the effect of species, leaf age, and time on area-based photosynthesis (P_N), stomatal conductance (g_s), maximum velocity of carboxylation (V_{cmax}), rate maximum of electron transport (J_{max}), rate of triose phosphate use (TPU) and CO_2 compensation point (Γ). The probability of type-I error (p -value), the F -statistics calculated using Wilks's criterion, the associated effect and error degrees of freedom, and the degrees of freedom of the effect and error is shown.

Effects and interactions	F -statistics	Degrees of freedom (effect, error)	p -value
Species	2.13	(7, 61)	0.049
Leaf age	2.43	(7, 61)	0.029
Time	1.62	(63, 350)	0.003
Species vs. leaf age	2.44	(7, 61)	0.028
Species vs. time	1.57	(63, 350)	0.006
Leaf age vs. time	0.76	(63, 350)	0.902
Species vs. leaf age vs. time	0.81	(63, 350)	0.840

Contrary to what was observed for P_N , the CO_2 compensation point (Γ) was on average significantly higher in the new leaves ($4.68 \pm 0.08 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$) than in the OL ($3.90 \pm 0.04 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$), for both species (Fig. 7). The average Γ obtained for *C. americana*, regardless of other factors (leaf age and month), was $4.10 \pm 0.06 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$, lower than that found for *V. divergens* ($4.57 \pm 0.06 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$). For *C. americana*, Γ differed significantly between the dry and flood seasons, decreasing from 4.5 to $3.7 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ when averaged across the leaf age. For *V. divergens*, seasonal differences for Γ were less pronounced, ranging from $4.5 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$ to $4.0 \mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$; however, Γ of the YL was highest during the dry-wet season transition in September–October 2009 (Fig. 7).

Tissue N and P concentrations varied significantly over time, and while the YL had generally higher N and P concentration, there were no significant differences

between the YL and OL or species (Fig. 5). Leaf N and P concentrations for both species increased during the dry season and reached a peak in September; the old *V. divergens* leaves exhibited lower seasonal variation in N concentration than the old *C. americana* leaves (Fig. 5). However, the YL appeared to have larger variations over time than the OL, and on average, seasonal variations in the N and P concentration of the young *V. divergens* leaves were larger than that observed for the young *C. americana* leaves.

Functional relationships between V_{cmax} , J_{max} , P_N , SLA, N, and P concentration: Stepwise linear regression revealed significant relationships between V_{cmax} , J_{max} , P_N , SLA, and leaf N and P concentration (Fig. 8, Table 2). A strong relationship between J_{max} and V_{cmax} was observed regardless of species and the leaf age ($r^2 = 0.90$, $p < 0.0001$, Table 2), and as expected, these two parameters were

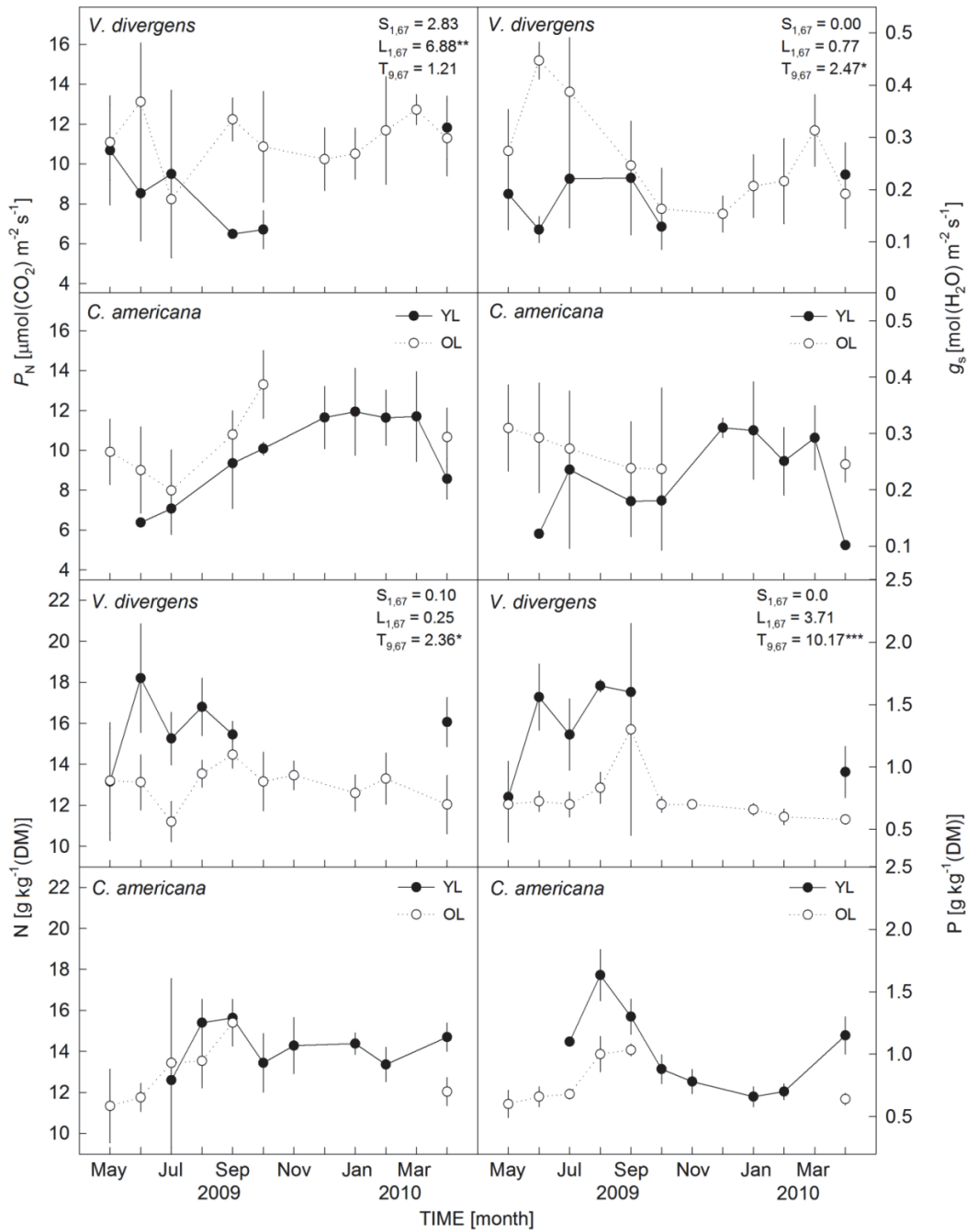


Fig. 5. Area-based leaf photosynthesis (*left top panels*), stomatal conductance (*right top panels*), leaf nitrogen (*left bottom panels*) and phosphorus (*right bottom panels*) concentration for young (YL, *solid circles, solid lines*) and old (OL, *open circles, dotted lines*) leaves of *V. divergens* (*top panels*) and *C. americana* (*bottom panels*) during the May 2009–April 2010 study period. Also shown are the results of a 3-way ANOVA (*F*-statistics and degrees of freedom) for the effects of species (S), leaf age (L) and season (T). Mean \pm SE (*n* = 5). **p*<0.05; ***p*<0.01; ****p*<0.001.

strongly related to P_N . V_{cmax} , J_{max} , P_N , and SLA varied significantly as a function of the leaf age (Table 2). Also as expected, these variables were strongly related to the leaf N and P concentration (Fig. 8, Table 2). J_{max} generally demonstrated a stronger relationship to N and P concentration than V_{cmax} ; however, the effect of N on J_{max}

was significantly affected by the leaf age. P_N , as a dependent variable, showed positive relations with N, P, and SLA (r^2 ranged between 0.12 and 0.54; Table 2) and weak or nonexistent relationships with the YL. N concentration was not associated with SLA and it was weakly related to P (Table 2).

Discussion

The physiological performance of *V. divergens* and *C. americana* was complex and varied depending on surface hydrology and leaf phenology. There were large differences in the timing and duration of leaf production, with *V. divergens* retaining leaves longer than *C. americana*, but *C. americana* producing leaves for a longer period of time. This implied that the leaf turnover was lower and/or more stable in *V. divergens* than *C. americana*. The OL of *V. divergens* had also a lower and more stable SLA than the YL leaves, implying a higher and more consistent allocation of C to maintain a leaf structure (Vitousek 1984, Reich *et al.* 1998, Baruch and Goldstein 1999, Gulias *et al.* 2003). However, SLA was similar both for *V. divergens* and *C. americana*.

Values of V_{cmax} and J_{max} for both species were within the ranges reported for other tropical tree species (Carswell *et al.* 2000, Coste *et al.* 2005, Domingues *et al.* 2005, Kenzo *et al.* 2006). *V. divergens* appeared to have consistently higher rates of V_{cmax} and J_{max} than *C. americana*, which partially confirmed our hypothesis. These results indicated that *V. divergens* had a greater ability to adjust photosynthetic capacity to variations in hydrology. Even so, while species differences in V_{cmax} and J_{max} were statistically significant, they were apparently not strong enough to cause significant differences in P_{N} . *V. divergens* also had a consistently higher Γ than *C. americana*,

which implied a higher respiratory CO_2 cost (Smith *et al.* 1976). Such an increase in the respiratory cost for *V. divergens* would be expected to compensate for the higher photosynthetic capacity (V_{cmax} and J_{max}), which could explain in part the similarity in P_{N} between *V. divergens* and *C. americana*.

The fact that P_{N} was similar for both species over seasonal time scales was striking, given the large fluctuations in surface hydrology. These data suggest that both *V. divergens* and *C. americana* are tolerant of seasonal flooding. This was expected with *V. divergens*, which is native to seasonally flooded tropical rainforest of the Amazon Basin (Pott and Pott 1994, Nunes da Cunha and Junk 2004). This behavior, found for *C. americana*, is similar to many species of Amazonian floodplains that are capable of growing in upland and flooded forests (Maurenza *et al.* 2011). However, *C. americana* is native to well-drained cerrado soils (Medina and Francisco 1994, Lorenzi 2002), and therefore, it was expected that the P_{N} of *C. americana* should be more variable over seasonal time scales and substantially lower than *V. divergens* during flooding. However, according to Parolin *et al.* (2010b), tolerance to drought stress has many of the same anatomical and physiological features that help plants tolerate the effects of flooding. Therefore, this tolerance might partly explain

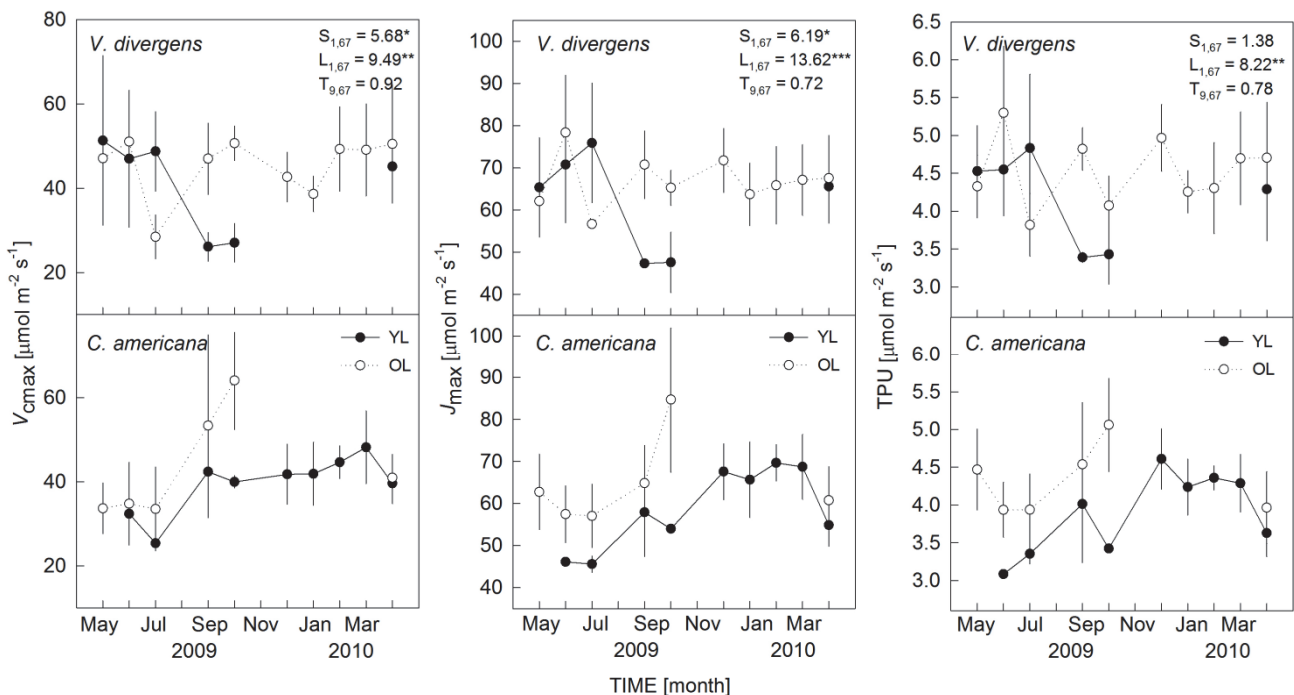


Fig. 6. Area-based maximum rate of carboxylation (*left panels*), electron transport (*middle panels*) and triphosphate utilization (*right panels*) for young (YL, *solid circles, solid lines*) and old (OL, *open circles, dotted lines*) leaves of *V. divergens* (*top panels*) and *C. americana* (*bottom panels*) during the May 2009–April 2010 study period. The results of a 3-way ANOVA (F -statistics and degrees of freedom) for the main effects of species (S), leaf age (L) and season (T) are also shown. Mean \pm SE ($n = 5$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

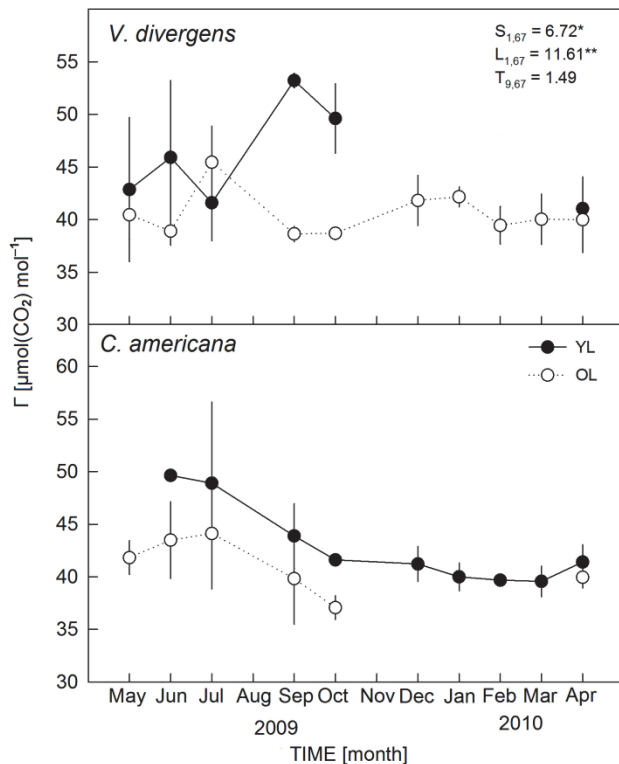


Fig. 7. CO_2 compensation point for young (YL, solid circles, solid lines) and old (OL, open circles, dotted lines) leaves of *V. divergens* (top panel) and *C. americana* (bottom panel) during the May 2009–April 2010 study period. The results of a 3-way ANOVA (F -statistics and degrees of freedom) for the main effects of species (S), leaf age (L) and season (T) are also shown. Mean \pm SE ($n = 5$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the invasion and spread of *C. americana* in Brazilian Pantanal.

The seasonal consistency in P_N occurred despite a significant seasonal variation in g_s . For example, g_s in both species tended to be higher during the dry season, which suggested that flooding limited g_s . As flooding ensues, soil becomes hypoxic or anoxic (Kozlowski 1984), and there is often a decline in g_s (Maurenza *et al.* 2009) due to a variety of mechanisms, including a reduction in ATP supply (Epstein and Bloom 2005), the buildup of lactic acid and cytoplasm acidification (Crawford 1992, Drew 1997, Oliveira and Joly 2010), a reduction in permeability and hydraulic conductivity of the roots, and an increase in abscisic acid (ABA) synthesis (Wilkinson and Davies 2002). While it is unknown, which of the above mechanisms was the most important in limiting g_s , such limitations may be considered a survival mechanism to flooding, because water loss from transpiration cannot be counterbalanced by root water absorption (Mielke *et al.* 2003, Dalmolin *et al.* 2012).

Dry season rates of P_N , g_s , V_{cmax} , and J_{max} were often higher than the wet season rates, indicating the water

sufficiency during the dry season. Surface soil water content is low during the dry season; however, there is an evidence that *V. divergens* and *C. americana* have an access to deep water sources during the dry season (Haase 1999, Biudes *et al.* 2008, Sanches *et al.* 2011, Vourlitis *et al.* 2011). The fact that both species initiated a leaf production during the dry season is a strong indication that they are able to use water from deeper soil layers (Medina 1982, Sarmiento 1984, Goldstein *et al.* 1989, Medina and Francisco 1994).

Some of the more consistent differences in gas exchange variables were due to the leaf age. The OL had higher rates of P_N than the YL, which may seem counter-intuitive, given the higher SLA of the YL (Reich *et al.* 1998, Baruch and Goldstein 1999, Gulias *et al.* 2003). Generally, leaves with a lower SLA (the OL for the species observed here) have a lower rate of P_N per N unit (Field and Mooney 1986), because thicker leaves cause internal shading and increase resistance to CO_2 (Terashima and Hikosaka 1995). However, our data indicate the opposite, the OL had a higher rate of P_N despite having slightly lower tissue N concentration, suggesting that shading and/or diffusion limitations were minimal for both *V. divergens* and *C. americana*. Indeed, the leaf age was an important factor for V_{cmax} , J_{max} , and TPU, indicating that the older leaves had a higher photosynthetic capacity than the YL, especially for *V. divergens*. In contrast, the YL had a consistently higher Γ than the OL, which is consistent with the observation of an increase in photosynthetic capacity relative to respiration as leaves age (Smith *et al.* 1976).

V. divergens and *C. americana* showed strong relationships between mass-based P_N , V_{cmax} , J_{max} , SLA, N and/or P. However, our data indicated that the V_{cmax} , J_{max} , and P_N for both species were relatively more affected by P than by N. This interpretation was supported by the fact that the relationships between these variables and P yielded the highest coefficient of determination (r^2) values and the highest slopes of the regression lines. The N/P mean ratio for *C. americana* and *V. divergens* leaves was 17.2 and 17.4, respectively, which slightly exceeds the threshold of 16 suggested by Aerts and Chapin (2000) as evidence of limitation by P. Vourlitis *et al.* (2011) reported that extractable P at the study site was approximately $2 \mu\text{g(P)} \text{ g}^{-1}$ (dry soil), and while this value is typical of many cerrado soils (Lopes and Cox 1977), such a low value indicates the potential for P limitation.

Low soil P can lead directly to P_i limitation in the chloroplast (Heldt and Rapley 1970), a decline in the synthesis of sucrose (Stitt *et al.* 1987), ATP and RuBP (Rao and Terry 1989), and a reduction in the photosynthetic capacity (Terry and Ulrich 1973). According to Adam *et al.* (2000), a limitation by TPU is not commonly observed in field experiments, but our data suggest that TPU limitation was important at high concentrations of C_i (Figs. 2,3). TPU estimates ranged from $4.41 \mu\text{mol m}^{-2} \text{ s}^{-1}$

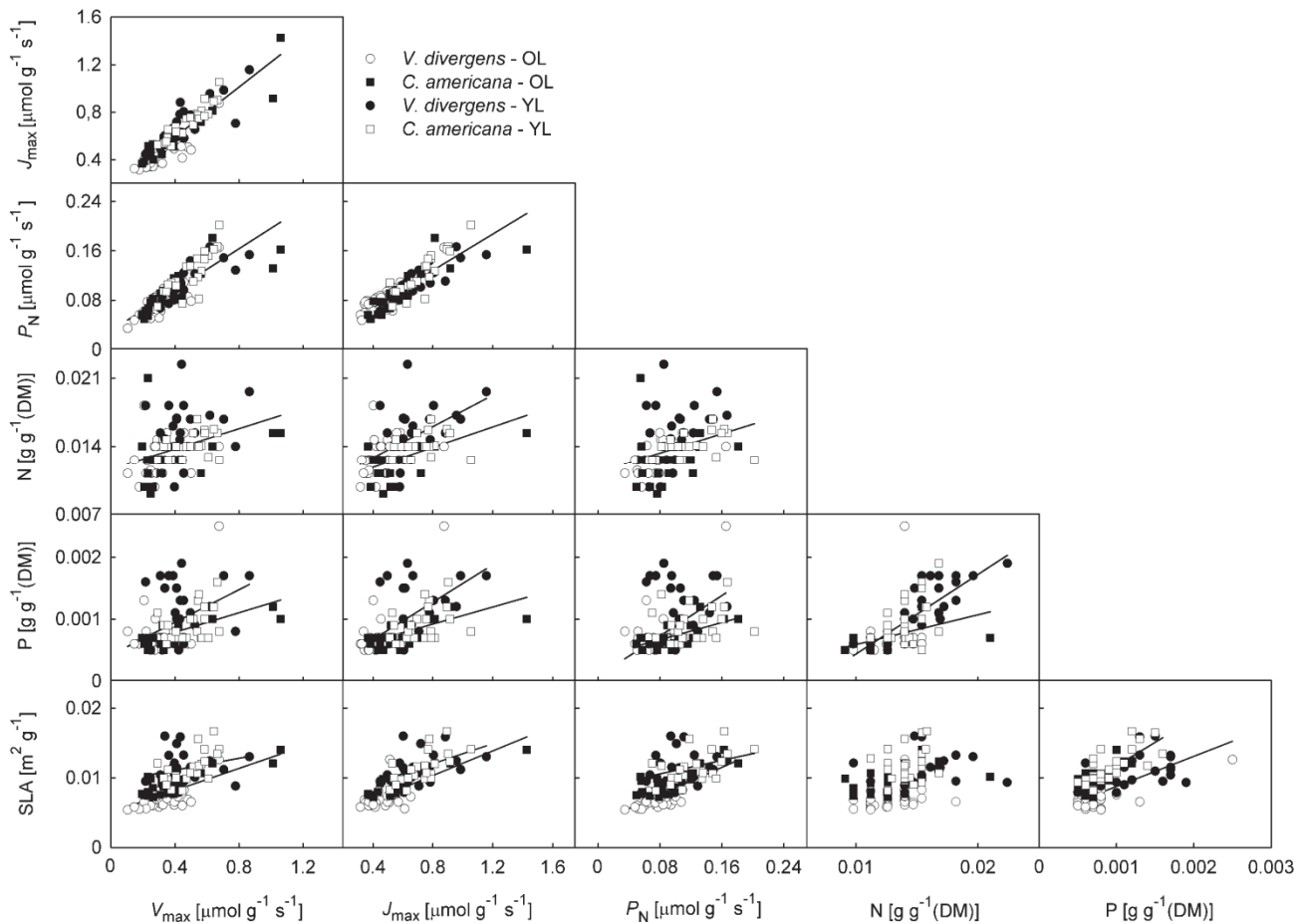


Fig. 8. Pairwise scatter plots of mass-based maximum rate of carboxylation (V_{cmax}), electron transport (J_{max}), photosynthesis (P_N), specific leaf area (SLA), P concentration (P), and leaf N concentration (N) for young (YL, closed symbols) and old (OL, open symbols) leaves for *V. divergens* (circles) and *C. americana* (squares) during the May 2009–April 2010 study period. Regression lines indicate significantly linear trends (see Table 2 for details).

for *V. divergens* and $4.12 \mu\text{mol m}^{-2} \text{s}^{-1}$ for *C. americana*, values very close to that found by Wullschlegel (1993) for another tropical tree species, *Tabebuia rosea* ($4.6 \mu\text{mol m}^{-2} \text{s}^{-1}$). Jacob and Lawlor (1991) showed that the decrease in the leaf photosynthetic capacity with an inadequate supply of phosphate was determined mainly by mesophyll factors and not by decreased stomatal conductance and a CO_2 diffusion restriction. This is consistent with our findings, since there was no significant change in the g_s over the months for both species, while a change was observed for V_{cmax} and J_{max} , suggesting a mesophyll limitation.

In conclusion, our results suggested that the flood-adapted *V. divergens* did not have a higher rate of leaf photosynthesis than *C. americana* as hypothesized. Rather, species differences were influenced by temporal variations in flooding and the leaf age. The OL tended to

have a higher photosynthetic capacity than the YL, especially for *V. divergens*, even though the older leaves had a significantly lower SLA. The leaf CO_2 assimilation and the photosynthetic capacity of both species were lower during the flood period, but differences were not statistically significant indicating that both species were able to tolerate flooding. Seasonal flooding significantly reduced g_s , but again, the decline in g_s did not appear to limit P_N . The physiological performance of both species was strongly related to the leaf N and P concentrations; however, P limitation appeared to be more important than N limitation for these species and ecosystem. Our results suggested that both species are tolerant to flooding even though they were adapted to very different hydrological conditions. Such physiological plasticity, especially for *C. americana*, might be a key feature for its ability to survive and persist in the seasonally flooded Pantanal.

Table 2. Linear regression relationships between mass-based maximum velocity of Rubisco for carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), photosynthesis (P_{N}), specific leaf area (SLA), P content (P) and leaf N content (N) for *V. divergens* (*Vg*) and *C. americana* (*Ca*) with different leaf ages (old leaves – OL, and young leaves – YL) in the Brazilian Pantanal. ns indicates relationships that are not statistically significant ($p > 0.05$), thus no slope or intercept is presented. Units: V_{cmax} , J_{max} , P_{N} : [$\mu\text{mol g}^{-1} \text{s}^{-1}$]; SLA: [$\text{m}^2 \text{g}^{-1}(\text{DM})$]; N and P: [$\text{g g}^{-1}(\text{DM})$].

Interactions	Variable Dependent	Independent	<i>n</i>	<i>r</i> ²	<i>p</i> -value	Slope	Intercept
Combined	V_{cmax}	J_{max}	100	0.90	<0.0001	1.2	12.12
Combined	V_{cmax}	P_{N}	104	0.69	<0.0001	0.2	0.03
Combined	V_{cmax}	N	103	0.13	<0.001	26.1	0.04
<i>Vg</i>	V_{cmax}	P	59	0.18	<0.001	139.9	0.24
<i>Ca</i>	V_{cmax}	P	44	0.35	<0.001	443.8	0.08
OL	V_{cmax}	SLA	60	0.49	<0.001	62.9	-0.14
YL	V_{cmax}	SLA	-	-	ns	-	-
Combined	J_{max}	P_{N}	100	0.77	<0.001	5.2	0.08
<i>Vg</i>	J_{max}	N	56	0.33	<0.001	41.6	-0.04
<i>Ca</i>	J_{max}	N	43	0.36	<0.001	68.1	-0.24
<i>Vg</i>	J_{max}	P	56	0.38	<0.001	253.1	0.32
<i>Ca</i>	J_{max}	P	43	0.29	<0.001	430.0	0.31
OL	J_{max}	SLA	58	0.69	<0.001	78.8	-0.10
YL	J_{max}	SLA	42	0.26	<0.001	35.4	0.31
Combined	P_{N}	N	104	0.12	<0.001	4.9	0.03
<i>Vg</i> – OL	P_{N}	P	38	0.32	<0.001	38.9	0.05
<i>Ca</i> – OL	P_{N}	P	22	0.44	<0.001	127.3	0.002
<i>Vg</i> – YL	P_{N}	P	-	-	ns	-	-
<i>Ca</i> – YL	P_{N}	P	-	-	ns	-	-
OL	P_{N}	SLA	60	0.54	<0.001	11.0	-0.002
YL	P_{N}	SLA	44	0.12	0.022	4.9	0.06
OL	N	P	70	0.13	0.002	2.6	0.01
YL	N	P	53	0.49	<0.001	3.8	0.01
<i>Vg</i> – OL	N	SLA	-	-	ns	-	-
<i>Ca</i> – OL	N	SLA	-	-	ns	-	-
<i>Vg</i> – YL	N	SLA	-	-	ns	-	-
<i>Ca</i> – YL	N	SLA	-	-	ns	-	-
<i>Vg</i>	P	SLA	67	0.52	<0.001	0.1	-0.001
<i>Ca</i>	P	SLA	52	0.58	<0.001	0.1	-0.001

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