

Do soil fertilization and forest canopy foliage affect the growth and photosynthesis of Amazonian saplings?

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ABSTRACT: Most Amazonian soils are highly weathered and poor in nutrients. Therefore, photosynthesis and plant growth should positively respond to the addition of mineral nutrients. Surprisingly, no study has been carried out *in situ* in the central Amazon to address this issue for juvenile trees. The objective of this study was to determine how photosynthetic rates and growth of tree saplings respond to the addition of mineral nutrients, to the variation in leaf area index of the forest canopy, and to changes in soil water content associated with rainfall seasonality. We assessed the effect of adding a slow-release fertilizer. We determined plant growth from 2010 to 2012 and gas exchange in the wet and dry season of 2012. Rainfall seasonality led to variations in soil water content, but it did not affect sapling growth or leaf gas exchange parameters. Although soil amendment increased phosphorus content by 60 %, neither plant growth nor the photosynthetic parameters were influenced by the addition of mineral nutrients. However, photosynthetic rates and growth of saplings decreased as the forest canopy became denser. Even when Amazonian soils are poor in nutrients, photosynthesis and sapling growth are more responsive to slight variations in light availability in the forest understory than to the availability of nutrients. Therefore, the response of saplings to future increases in atmospheric [CO₂] will not be limited by the availability of mineral nutrients in the soil.

Introduction

The Amazon rainforest is the largest tropical rainforest in the world. It is particularly important because of its prominent role in carbon and water cycles. Its vegetation stores about 90 Pg (1 Pg = 10¹⁵g) of carbon (Saatchi et al., 2007), and about 50 % of the rainfall in the region comes from water cycled via transpiration (Salati, 1987; Fisher et al., 2009). Net productivity of tropical forests may be limited by several factors, including nutrient deficiency (Vitousek et al., 2010), sunlight (Graham et al., 2003) and soil water content (Wagner et al., 2012).

Because most Amazonian soils are highly weathered and poor in nutrients (Sanchez, 1976), it seems possible that phosphorus (P) availability, for example, can limit forest productivity in the Amazon (Harrington et al., 2001; Townsend et al., 2007, Vitousek et al., 2010; Townsend et al., 2011). Nevertheless, the real effect of P on net primary production still remains to be determined, as tropical forests have enough labile phosphorus to account for P requirements to sustain plant growth (Johnson et al., 2003). Furthermore, calcium (Ca) (Paoli and Curran, 2007) and potassium (K) may also limit tree growth or fine root turnover in tropical rainforests (Kaspari et al., 2008; Yavitt et al., 2011).

Although there are uncertainties about how Amazonian trees respond to nutrient addition, to our knowledge no study has been carried out *in situ* in the central Amazon to address this issue for juvenile trees. In the rainforest of Panama, addition of mineral fertilizers may increase sapling growth of *Tetragastris panamensis* (Yavitt and Wright, 2008), and in a secondary forest of Hawaii, *Acacia koa* trees may grow faster in P-amended soils (Scowcroft et al., 2007).

In addition to nutrients, water availability can be responsible for over 60 % of the environmental effect on tree growth (Wagner et al., 2012). In this study, we hypothesized that the growth of saplings in the forest understory is limited by both sunlight and the availability of soil nutrients, and that variations in soil water content across rainfall seasons influence photosynthesis and sapling growth. Thus, we evaluated how photosynthetic rates and growth of saplings respond to the addition of mineral nutrients, the variation in leaf area index (LAI) of the forest canopy and to changes in soil water content associated with rainfall seasonality.

Materials and Methods

Study site and plant materials

The study was conducted 60 km north of Manaus (02°36'21" S; 60°08'11" W), state of Amazonas, Brazil, in an area of native "terra-firme" forest. The predominant soil type is an acidic clayey textured Oxisol. The region has a humid equatorial climate, with a mild short dry season (July-Sept, rainfall of 50-100 mm per month), and a dry-wet transition month (Oct). The wet season extends from Nov to May (200-300 mm monthly); annual rainfall is 2,240 mm and the mean annual temperature is about 27 °C.

In this study, we used saplings (1.0 to 3.0-m tall) from five tree species (*Minquartia guianensis* Aubl., Olacaceae; *Guatteria olivacea* R.E.Fr, Annonaceae; *Rinorea guianensis* Aubl., Violaceae; *Simarouba amara* Aubl., Simaroubaceae, and *Duroia macrophylla* Huber, Rubiaceae), selected because of their shade tolerance and the relative abundance of saplings in the forest understory.

Five plants per species and mineral nutrient treatments were tested, except for *D. macrophylla*, in which only four replications were available for the soil amendment treatment (the 5th plant was badly injured before data collection).

Physical environment

Photosynthetic photon flux (PPF), air temperature, air relative humidity (RH) and precipitation above the forest canopy were measured at the nearest open place, the top of a 40-m-tall observation tower, 3.3 km away from the study site. Overstory PPF data were recorded at the observation tower (PPF_{open}) at 15-min intervals with a quantum sensor (Li-190 SA, Li-Cor, NE, USA) connected to a data logger (Li-1400, Li-Cor, NE, USA). Air temperature and RH were logged (Humitter 50Y, Vaisala Oy, Finland) at 30-min intervals, at the same place. In addition, light data in the forest understory (PPF_{und}) were also recorded at the study site.

Soil amendment, soil water content and leaf area index

We applied 75 g of a slow-release fertilizer (containing 15 % N, 9 % P, 12 % K, 1 % Mg, 2.3 % S, 0.05 % Cu, 0.45 % Fe, 0.06 % Mn and 0.02 % Mo) per plant. The control was unfertilized plants. In the fertilized treatment, the total amount of fertilizer was equally split into five applications (Dec 2010, Mar, June, Sept and Dec of 2011; i.e. 15 g per plant on each occasion). The fertilizer was applied from 30 to 50 cm of the stem base and 10-cm depth from the soil surface, forming a circle around the plant (soil amendment circle). The radius of this circle resembled that of the crown of each sapling. It is estimated that the radius of a plant root system corresponds to about two times its foliage radius (Gilman et al., 1987); thus, all nutrients applied were within the influence of the root zone.

To assess the effect of soil water content on photosynthesis, in the dry and rainy season of 2012, we determined soil water content (the ratio of soil water to dry soil mass) in four soil samples per plant microsite to obtain a mean. Each sample was collected at a depth of 10-100 mm. Soil water tension was estimated using the Van Genuchten (1980) equation with parameters obtained at the same Experiment Station by Ferreira et al. (2002). In addition, during the collection of photosynthesis data, predawn leaf water potential (Ψ_{leaf}) was determined (4:00 to 05:00) using a pressure chamber (1505D, PMS Instrument Company, Albany, OR, USA).

Soil samples (four subsamples per plant microsite mixed together to obtain a compound sample for analysis) were taken in Dec 2012 in the control and in the fertilized treatments 20 to 40 cm (depending on plant size) away from the stem base and at 10-cm depth from the soil surface for chemical and physical analyses. The double-acid Mehlich-I solution (0.05 M HCl + 0.0125 M H₂SO₄) was used to extract phosphorus (P), potassium (K), zinc (Zn), and iron (Fe). Calcium (Ca), mag-

nesium (Mg) and aluminum (Al) were extracted with 1M KCl (1:10 soil solution ratio). Mehlich-I-extractable phosphorus was determined using ammonium molybdate and the absorbance recorded at 660 nm (Shimadzu UVmini-1240, Shimadzu Corp., Kyoto, Japan); nitrogen was assessed using the Kjeldahl method. Concentrations of K, Ca, and Mg were determined by atomic absorption spectrophotometry (PerkinElmer 1100B; PerkinElmer Inc., Waltman, MA, USA), whereas Al in the extractant was measured by titration with 0.025 M NaOH. Soil texture was determined using the classic pipet method, which depends on the precipitation velocity of soil particles (Stokes' law) in a sedimenting soil suspension (Day, 1965). Soil samples were taken within the amendment circle, but 10 cm closer to the stem from the fertilization line to avoid sampling over the same place where the fertilized treatment had been applied.

The leaf area index (LAI, total one-side leaf area per unit ground surface area) was obtained both in the dry and rainy season of 2012 to estimate the canopy foliage above saplings. LAI was determined with a canopy analyzer (LAI-2000 Plant Canopy Analyzer, Li-Cor, NE, USA) using two synchronized sensors. One sensor was used to collect data at the forest understory (six readings, forming a circle around each sapling) and the second, operating in the remote mode was installed on the top of the observation tower, to log LAI values above the forest canopy.

Leaf traits and tree growth

Gas exchange parameters were measured with an infrared gas analyzer (Li-6400, Li-Cor, NE, USA) in ten saplings per species (fertilized and unfertilized plants) and two leaves per plant, both in the wet and dry season of 2012. Data were collected at ambient conditions of temperature (28 ± 1 °C) and relative humidity (70 ± 5 %). Light response curves (A/PPF) were generated at a [CO₂] of 380 $\mu\text{mol mol}^{-1}$. The A/PPF light response curve was used to determine light saturated photosynthesis (A_{max} at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and g_s at light saturation ($g_{s,max}$).

The maximum carboxylation velocity of Rubisco (V_{cmax}) and the maximum rate of electron transport (J_{max}) were calculated using Farquhar's model (Farquhar et al., 1980). V_{cmax} and J_{max} data were standardized to 25 °C (Nascimento and Marengo, 2013). V_{cmax} and J_{max} were obtained after measuring photosynthesis at CO₂ concentrations of 50 to 2000 $\mu\text{mol mol}^{-1}$ and saturating light intensity (1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Specific leaf area, SLA (the inverse of leaf dry mass per unit area, LMA) and leaf thickness (LT) were also determined in the same leaves used for photosynthetic measurements. SLA was determined as the leaf area to leaf mass ratio in two leaves per plant. Leaf dry mass was obtained after oven drying at 72 °C until constant mass. LT was measured with digital calipers (accuracy of 10 μm) in 240-mm²-leaf disks (two per leaf). We also

calculated leaf longevity (in months) as LMA (in g m^{-2}) divided by 5.128; Wright et al., 2004). Light saturated photosynthesis on a mass basis (A_{mass} , $\text{nmol g}^{-1} \text{s}^{-1}$) was obtained as A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) multiplied by SLA ($\text{m}^2 \text{kg}^{-1}$).

Height (H) and diameter (D) of saplings were recorded at 30-d-intervals for 24 months (Dec-2010 to Dec-2012). Plant height was measured from the base of the plant to the apex. Stem diameter was measured (mean of two measurements taken at right angles from each other) with digital calipers at 50 cm from the soil surface. During this period, monthly increase in diameter (ΔD) and height (ΔH) were determined as the difference in diameter (or height) between two consecutive measurements. Also, we calculated the annual relative growth rate (RGR, the annual biomass gain to initial biomass ratio) as $(B_{\text{tf}} - B_{\text{ti}})/B_{\text{ti}}$, where B_{tf} and B_{ti} stand for the biomass of saplings at the end (t_f) and the beginning of the experiment (t_i).

Biomass per unit volume depends on wood density, which often varies among species (Nogueira et al., 2007). As there is a lack of information about the wood density of saplings, the equation of Cummings et al. (2002), which disregards wood density in calculation, was used to estimate the biomass of saplings (B), as follows: $B = [(\exp(1.0583 \times \ln((D/10))^2 + 4.9375)) \times 1.143]$. In this equation B is in gram and D in millimeter.

Statistical analyses

Data were analyzed using the two-way repeated measures analysis of variance (ANOVA) procedure, for a completely randomized design with five replications (saplings). The repeated measures analysis was performed because the same set of plants was assessed in both rainfall seasons. The effects of LAI and other study variables on growth and photosynthetic parameters were examined by regression analysis. Sigma Plot 11.0 (Systat Software, Inc, USA) was used for statistical analyses.

Results

Physical environment

During the study period monthly rainfall ranged from 41 mm in Sept 2011 to 557 mm in Feb 2012 (Figure 1A). Above the forest canopy mean air temperature was 24.9°C , and monthly mean PPF varied from 20.9 to $32.6 \text{ mol m}^{-2} \text{ day}^{-1}$ (Figure 1B, C), with a mean maximum of $1084 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at noon (inset in Figure 1C). In the forest understory, the mean temperature was lower than the air temperature observed above the forest canopy, and light intensity did not exceed 1-2 % of PPF values recorded above the forest canopy (Figure 1B, C).

PPF and air temperature values recorded in this study are similar to those reported previously for the same region (Nascimento and Marenco, 2013). Shuttleworth et al. (1984) also found that radiation above the canopy is only 40 % of extra-terrestrial radiation, about

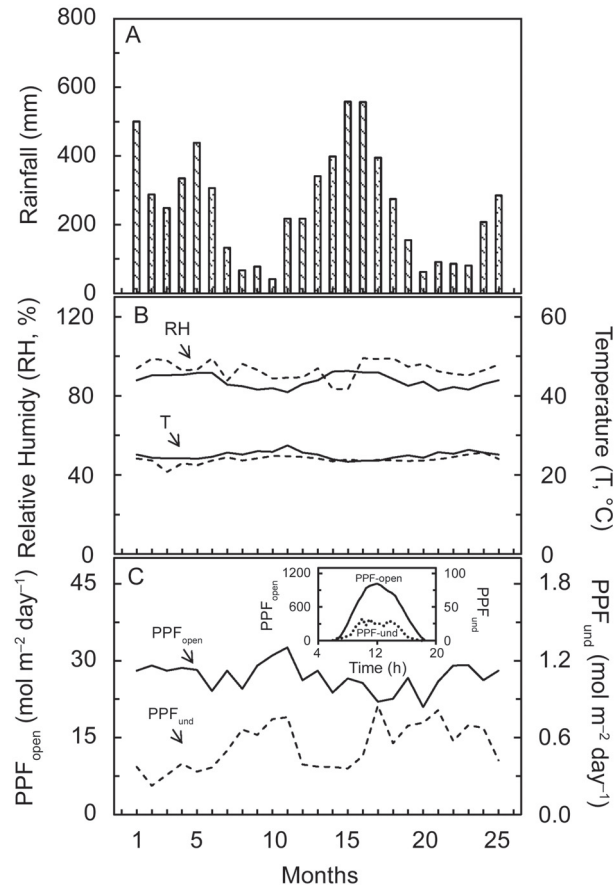


Figure 1 – Monthly rainfall (A), mean air temperature (T) and mean air relative humidity (RH) above the forest canopy (solid line), panel B; T and RH at the forest understory (dashed line) and mean photosynthetic photon flux above the forest canopy (PPF_{open}) and PPF at the forest understory (PPF_{und}) at the study area, panel C. The inset in panel C shows mean values of PPF_{open} and PPF_{und} (in $\mu\text{mol m}^{-2} \text{ s}^{-1}$) as a function of time. Data were collected from Dec-2010 to Dec-2012. In the x-axis, the numerals correspond to the months, beginning in Dec 2010 (month 1) and ending in Dec 2012 (month 25).

450 W m^{-2} (i.e. about $930 \mu\text{mol m}^{-2} \text{ s}^{-1}$), very close to PPF values observed in this study. Rainfall seasonality led to variations in soil water content, from 51 % (soil water tension of about 95 kPa) in the dry season to 67 % in the rainy season, soil water tension of about 10 kPa ($p < 0.05$, t test). Also, it led to changes in predawn leaf water potential (Ψ_{leaf}) from -0.26 MPa (dry period) to -0.13 MPa in the rainy season.

As a result of soil amendment, Mehlich-extractable P increased from 0.84 to 1.35 mg kg^{-1} (i.e. an increase of 60 %, Table 1). Despite the amount of fertilizer applied (100 g m^{-2}), P was the sole element that had an increase worthy of note in response to soil amendment. This suggests that (except for P) the amount of added elements was not high enough to affect the availability of soil nutrient contents at the plant microsites.

Leaf traits and sapling growth

Although there was variation in soil water content and Ψ_{leaf} across rainfall season, neither sapling growth rates nor the leaf traits (photosynthetic parameters, LT, SLA, and the relative chlorophyll content) were affected by rainfall seasonality ($p > 0.05$). Also there was no effect of soil amendment on either sapling growth or leaf traits, including photosynthesis, $g_{s\text{-max}}$, V_{cmax} , J_{max} or SPAD values ($p > 0.05$). Thus, data obtained across seasons and fertilization treatments (with and without soil amendment) were pooled to examine interspecific variation and the effect of leaf area index on sapling growth and parameters of photosynthesis.

Interspecific variation in leaf traits and sapling growth

Light saturated photosynthesis, $g_{s\text{-max}}$, V_{cmax} and J_{max} differed among species, and across species A_{max} tended to increase in response to stomatal opening (Table 2). We observed little variation in the relative chlorophyll content and, on average, SPAD value was 52.7 across species. Although Ψ_{leaf} varied within species, changes in leaf water content did not lead to variation

in $g_{s\text{-max}}$ or A_{max} . V_{cmax} and J_{max} ranged from 18 to 29 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 40 to 59 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In comparison with LT, SLA varied only within a narrow range, from 12.2 to 17.7 $\text{m}^2 \text{kg}^{-1}$ (Table 2). A_{max} ranged from 3 to 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and mean $g_{s\text{-max}}$ was 0.1 $\text{mol m}^{-2} \text{s}^{-1}$.

Across species, A_{mass} ranged between 32 and 146.1 $\text{nmol g}^{-1} \text{s}^{-1}$ (mean of 62.2 $\text{nmol g}^{-1} \text{s}^{-1}$) and mean LMA was 67.6 g m^{-2} (range of 41 to 95.6 g m^{-2}), demonstrating positive correlation between LT and LMA ($r = 0.44$; $p < 0.01$). On average, estimated leaf longevity was 13.2 months. Although there was no correlation between A_{max} and LMA ($p > 0.05$), the relationship between A_{mass} and LMA was highly significant ($p < 0.01$, Figure 2A). Leaf water potential varied from -0.24 MPa in *S. amara* to -0.16 MPa in *M. guianensis*. In addition, A_{max} (but not A_{mass}) and $g_{s\text{-max}}$ were responsive to variation in LT (Figure 3A, C). We found positive correlation between V_{cmax} or J_{max} and $g_{s\text{-max}}$ (Figure 3B, D). Monthly increase in diameter (ΔD) ranged from 0.08 (*G. olivacea*) to 0.17 mm in *S. amara*, which also showed the highest ΔH value (Table 2).

Table 1 – Chemical and physical properties of the fertilized and the unfertilized soil at the study area. Mean and standard deviation (within parentheses).

Parameter or element	unfertilized soil	Fertilized soil
Mehlich-extractable P (mg kg ⁻¹)	0.84(0.31) b	1.35(0.64) a
Total N (g kg ⁻¹)	2.17(0.40) a	2.25(0.26) a
pH (1 M KCl)	3.85(0.12) a	3.88(0.09) a
pH (H ₂ O)	3.97(0.15) a	3.92(0.13) a
K (mg kg ⁻¹)	29.10(4.29) a	33.38(11.01) a
Ca (mg kg ⁻¹)	13.40(6.09) a	13.53(8.65) a
Mg (mg kg ⁻¹)	14.48(2.40) b	16.93(4.87) a
Fe (mg kg ⁻¹)	157.58(32.95) a	152.55(39.69) a
Zn (mg kg ⁻¹)	0.80(0.23) a	0.81(0.23) a
Al (mmol ⁺ kg ⁻¹)	19.0(4.0) a	19.2(2.9) a
Sand (g kg ⁻¹)	112	-
Silt (g kg ⁻¹)	150	-
Clay (g kg ⁻¹)	738	-

Within lines means followed by the same letter do not differ (Student's t test, $p \leq 0.05$).

Table 2 – Light-saturated photosynthesis (A_{max}), stomatal conductance ($g_{s\text{-max}}$), maximum carboxylation velocity of Rubisco (V_{cmax}), maximum electron transport rate (J_{max}), monthly increase in diameter (ΔD) and height (ΔH), specific leaf area (SLA), leaf thickness (LT), relative chlorophyll content (SPAD values), leaf area index (LAI), and leaf water potential (Ψ_{leaf}) in five Amazonian saplings species. Each value represents the mean of ten plants per species across rainfall seasons.

Species	A_{max}	V_{cmax}	J_{max}	$g_{s\text{-max}}$	ΔH	ΔD	LT	LAI	SPAD	SLA	Ψ_{leaf}
	$\mu\text{mol m}^{-2} \text{s}^{-1}$			$\text{mol m}^{-2} \text{s}^{-1}$	mm		unitless		$\text{m}^2 \text{kg}^{-1}$		MPa
<i>M. guianensis</i>	3.37 b	21.79 b	49.53 bc	0.06 c	3.0 b	0.10 ab	0.14 b	4.66 a	52.3 a	15.86 ab	-0.16 a
<i>G. olivacea</i>	4.20 b	21.37 b	43.63 cd	0.12 ab	4.0 ab	0.08 b	0.15 b	4.69 a	53.7 a	13.41 cd	-0.19 ab
<i>R. guianensis</i>	4.09 b	23.25 b	52.22 b	0.08 bc	4.0 ab	0.11 ab	0.19 a	4.29 ab	54.8 a	14.39 bc	-0.17a
<i>S. amara</i>	5.79 a	29.11a	59.24 a	0.15 a	12.0 a	0.17 a	0.18 a	4.01 b	49.0 a	17.73 a	-0.24 b
<i>D. macrophylla</i>	4.04 b	18.28 b	40.27 d	0.10 abc	9.0 ab	0.12 ab	0.20 a	4.39 ab	54.1 a	12.26 d	-0.21ab
Mean	4.31	22.86	49.13	0.11	6.0	0.12	0.18	4.41	52.7	14.8	-0.21

Within columns, means followed by the same letter do not differ (Tukey test, $p \leq 0.05$).

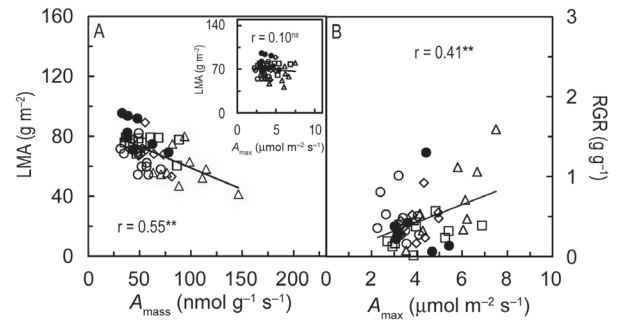


Figure 2 – Relationship between leaf dry mass per unit area (LMA) and light saturated photosynthesis on a mass basis, A_{mass} (A), and between annual relative growth rate (RGR) and light-saturated photosynthesis, A_{max} (B) in *Minuartia guianensis* (circle, ○), *Guatteria olivacea* (square, □), *Rinorea guianensis* (diamond, ◇), *Simarouba amara* (triangle, Δ) and *Duroia macrophylla* (solid circle, ●). The inset in panel A shows the relationship between LMA and A_{max} . Each symbol represents one plant per species and two leaves per plant. ns: not significant; ** significant at $p \leq 0.01$.

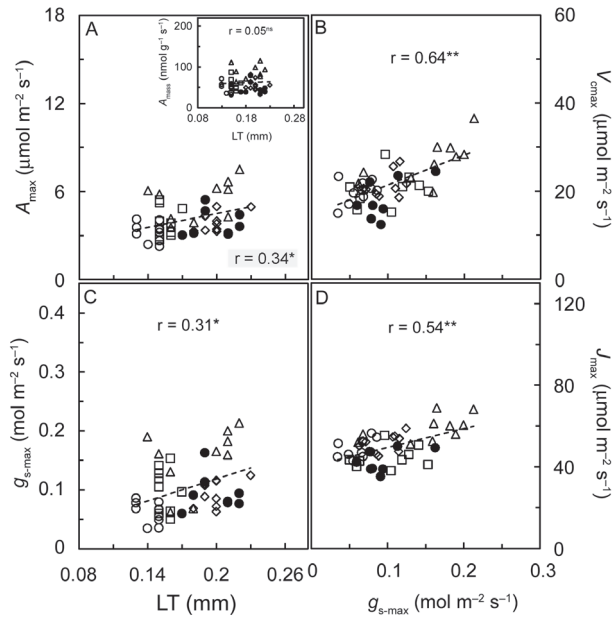


Figure 3 – Relationship between light-saturated photosynthesis (A_{\max}) and leaf thickness, LT (A); maximum carboxylation velocity of Rubisco (V_{cmax}) and stomatal conductance, $g_{\text{s-max}}$ (B); $g_{\text{s-max}}$ and LT (C); and $g_{\text{s-max}}$ and maximum electron transport rate, J_{max} , (D) in *Minuartia guianensis* (circle, ○), *Gutteria olivacea* (square, □), *Rinorea guianensis* (diamond, ◇), *Simarouba amara* (triangle, Δ) and *Duroia macrophylla* (solid circle, ●). The inset in panel A shows the relationship between A_{mass} and LT. Each symbol represents one plant per species and two leaves per plant. *significant at $p \leq 0.05$; **significant at $p \leq 0.01$.

Leaf traits and sapling growth in response to variation in leaf area index

LAI values did not differ between the dry and rainy season ($p > 0.05$) and across plant microsites they ranged from 4 to 4.7 (Table 2). LT, V_{cmax} and J_{max} were responsive to variation in leaf area index ($p \leq 0.05$, Figure 4A, C, E). RGR, A_{max} and $g_{\text{s-max}}$ declined with increases in LAI (Figure 4B, D, F). Finally, we found positive correlation between A_{max} and annual RGR ($p \leq 0.01$, Figure 2B).

Discussion

Effect of nutrient addition

Although the amount of nutrient added was relatively high (about 15 g m^{-2} of N and 9 g m^{-2} of P), no effect of soil amendment was observed on the parameters examined, which negates our initial hypothesis. The nil effect of the mineral nutrient treatments was unexpected because the highly-weathered Amazonian soils are of low fertility (Sanchez, 1976; Quesada et al., 2010). However, our results agree with Cleveland et al. (2011), who found only a weak correlation between P and net primary production. The absence of response to mineral fertilization suggests that either the length of

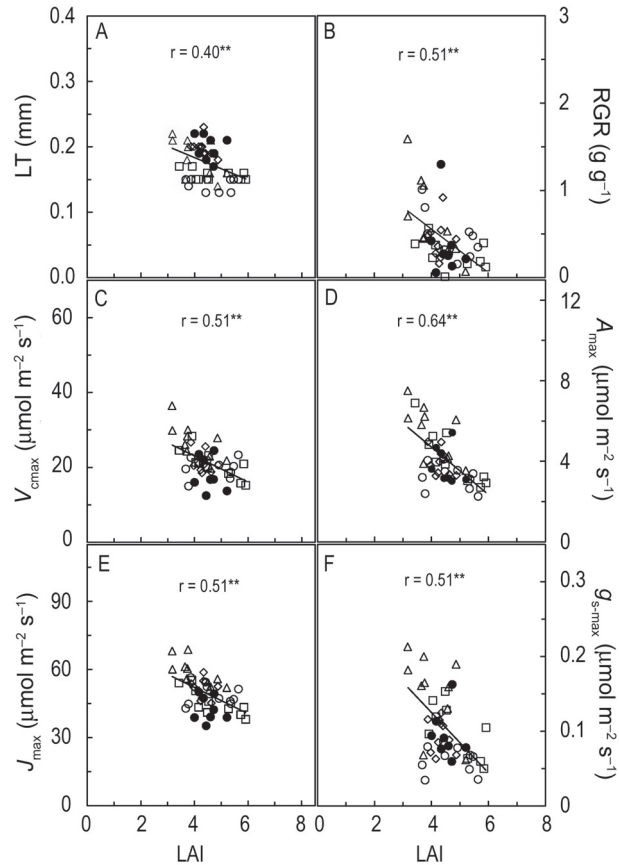


Figure 4 – Relationship between leaf area index (LAI) and leaf thickness, LT (A), annual relative growth rate, RGR (B), maximum carboxylation velocity of Rubisco, V_{cmax} (C), light-saturated photosynthesis, A_{max} (D), maximum electron transport rate, J_{max} , (E), stomatal conductance, $g_{\text{s-max}}$ (F) in *Minuartia guianensis* (circle, ○), *Gutteria olivacea* (square, □), *Rinorea guianensis* (diamond, ◇), *Simarouba amara* (triangle, Δ) and *Duroia macrophylla* (solid circle, ●). Each symbol represents one plant per species and two leaves per plant. **significant at $p \leq 0.01$.

the interval between nutrient addition (and thereby the total amount of nutrient added) was not short enough to affect the parameters examined or that the root system of neighbor canopy trees absorbed a large fraction of the nutrients supplied.

As the forest floor has high microbial activity, applied nitrogen can also be immobilized by soil microorganisms (Moreira et al., 2011), which makes it unavailable to plants. The lack of response to additional mineral N may also indicate the soil has a sufficient supply of this element, as a result of N-fixation by either symbiotic association or free-living microorganisms (Cleveland et al., 1999; Reed et al., 2010).

If sunlight availability is the primary factor restraining sapling growth at the forest understory (Ninemets, 2010; Gommers et al., 2013), increasing nutrient availability should only result in marginal (if any)

real enhancement in plant growth. Adaptation to low fertility soils is often accomplished by several mechanisms including enhanced nutrient use efficiency (e.g. recycling of litter nutrients, nutrient resorption from senescing leaves) and improved nutrient acquisition (e.g. phosphorous) by forming mycorrhizal association (Aerts and Chapin-III, 1999; Kobe et al., 2005).

Effect of water availability

No response of sapling growth and photosynthetic parameters to rainfall seasonality contradicts our expectation. Small changes in soil water content often lead to stomatal closure, and thereby to reduction in photosynthesis (Davies et al., 2002). Thus, absence of response to rainfall seasonality indicates that the decline in soil water content during the dry season was not large enough to affect stomatal functioning and carbon gain. Although soil water tension dropped in the dry season and led to variation in leaf water potential, soil water content was well above the permanent wilting point (lower limit of readily available water), about -1500 kPa for most plants (Van Genuchten, 1980).

Constancy of photosynthetic traits across seasons agrees with Mendes and Marengo (2010), who found higher photosynthetic rates only at midday rather than either early in the morning or in the afternoon. Rainfall events during the mild dry season and the little change in leaf area index across seasons contribute to maintain high soil water content, so that leaf water potential remains high, saving the leaves from water stress.

Plant growth in response to variation in forest canopy foliage

The mean growth rate observed is similar to that recorded in *Swietenia macrophylla* and *Minuartia guianensis* grown under shade condition (Azevedo and Marengo, 2012). This indicates that saplings used in the study (1 to 3-m tall) can take over a century to reach a minimum of 100 mm in diameter at breast height. LAI values found in this study are within the range (3.5 to 6) reported by others (Aragão et al., 2005; Malhado et al., 2009; Caldararu et al., 2012) for the Amazon region.

The effects of LAI (a measure of canopy foliage) on leaf traits can be explained on the ground that high LAI values lead to low light availability at the forest understory. Under shade conditions, leaf area per unit leaf dry mass tends to increase to enhance light capture, which ultimately results in thinner leaves (Valladares and Niinemets, 2008). In comparison with thicker leaves, thinner leaves have a lower concentration of photosynthetic proteins per unit leaf area (Hikosaka, 2004). This explains the reduction in photosynthetic traits and thereby, reduction in growth rates under denser canopy covers (higher LAI values; Kruger and Volin, 2006).

Leaf structure-function relationships

Our LMA values concur with those reported by Wright et al. (2004). LMA had negative correlation with

A_{mass} , but no correlation was found between A_{max} and LMA, which is in agreement with previous reports (Wright et al., 2004). A_{mass} often declines as LMA increases (Osnas et al., 2013), and in several species leaf longevity is greater in thick (high-LMA) leaves than in thinner ones, as an adaptive strategy to compensate for the high investment in constitutive defenses to avoid leaf damage (i.e. herbivory; Endara and Coley, 2011). The slight trend of A_{max} to respond to changes in LT but not to variation in LMA suggests that leaf density (the other component of LMA) negatively affects A_{max} , as previously reported for the A_{mass} /leaf density relationship (Niinemets, 1999).

As high-LMA leaves (thick leaves) are more expensive to produce, per area, they need to live longer in order to payback their construction costs (Osnas et al., 2013). In this study, estimated values of leaf life span are consistent with those reported by Williams et al. (1989), who observed that understory *Piper* species have leaf longevity greater than one year. Indeed, leaves with A_{mass} of $62.2 \text{ nmol g}^{-1} \text{ s}^{-1}$ (this study) have leaf longevity of 15 (Reich et al., 1991) to 30 months (Poorter and Bongers, 2006), which suggests our leaf longevity estimate is rather conservative. In comparison with gap-demanding species, shade tolerant plants also have high LMA, leaves with a long life span, and synthesize large amount of antiherbivore metabolites (Valladares and Niinemets, 2008; Gommers et al., 2013). However, contrary to expectations, they also have low phenotypic plasticity, perhaps because a large amount of assimilate is required to produce leaves able to withstand herbivory pressure (i.e. synthesis of allelochemicals) and other physical damage (Gommers et al., 2013).

Conclusions

Photosynthesis and sapling growth are most likely to be limited by light availability and light quality, rather than by the content of mineral nutrients. Decline in soil water content during the dry season does not seem to be large enough to reduce photosynthesis of understory saplings in central Amazonia. However, small changes in canopy openness influence growth and photosynthetic parameters of juvenile trees at the forest understory. Our results suggest that although the Amazonian soils are poor in nutrients, the response of saplings to future increases in atmospheric CO_2 concentration will not be limited by the availability of mineral nutrients in the soil.

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