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Photosynthesis-nitrogen relationships in pioneer plants of disturbed tropical montane forest sites

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Abstract

Tropical forest disturbances lead to the establishment of secondary successional plant communities constituted by light demanding species with high relative growth rate that conduct to rapid canopy closure. Two main strategies for N nutrition are: (*a*) mineral N acquisition in the form of NH_4 and NO_3, and (*b*) symbiotic atmospheric N_2 fixation. Given the high N requirement for maximization of leaf area and radiant energy absorption, we hypothesize that contrasting strategies of N nutrition in these environments are reflected in leaf photosynthetic characteristics. We compared the N-photosynthesis relationships and carbon balance parameters per unit leaf area as they vary with age in two species with contrasting N acquisition strategies: a N_2-fixer *Crotalaria anagyroides* HBK (*Papilionoideae*), and a mineral-N user *Verbesina turbacensis* HBK (*Asteraceae*). N_2 fixation capacity was associated to higher specific leaf area (SLA), higher photosynthetic capacity (P_{max}) per unit leaf area and leaf mass, and higher N content per unit leaf mass. The N_2-fixer species showed higher slope in the relationship P_{max}-N per unit leaf mass and area when compared to the leaves of non-fixer species. Moreover, the intrinsic photosynthetic N use efficiency (P_{max}/N) was higher in the N_2 fixer than in leaves of the non-fixer species. Changes in N due to leaf age resulted in larger changes in CO_2 flux density at the leaf level in the N_2-fixer species. The higher photosynthetic capacity of the N_2-fixer species was mechanistically related to higher stomatal conductance, internal CO_2 concentration (c_i) values closer to atmospheric CO_2 concentration (c_a), and lower intrinsic water use efficiency than the mineral N-user species. Despite their higher P_{max} per unit leaf area, total non-structural saccharides concentration was lower in mature leaves of the N_2-fixer plant as compared to the non-fixer counterpart. This might be caused by the presence of a larger root sink (symbionts) stimulating saccharides export and higher diurnal respiration rates.

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Additional key words: *Crotalaria anagyroides*; ecological succession; nitrogen use efficiency; respiration; saccharides; specific leaf area; stomatal conductance; *Verbesina turbacensis*; water use efficiency.

Introduction

Early successional habitats are characterized by high photosynthetically active radiation (PAR) reaching the soil surface, and relatively rapid changes in carbon to nutrient ratios associated to fluctuating microbial activity in the soil (Bazzaz and Picket 1980, Vitousek *et al.* 1989). High irradiances and transient increases in soil nutrient availability frequently favour the establishment of PAR demanding, fast growing plant species.

High growth rates usually lead to a rapid closure of the canopy that results in a strong competition for PAR. Individual traits leading to maximization of PAR interception at the canopy may confer advantage over competitors under such conditions. In pioneer species, this can be attained either by increasing the ratio of leaf area per unit plant mass, by increases in specific leaf area or by intrinsic photosynthetic capacity per unit leaf area, among others. The leaf area development ultimately depends on the uptake of mineral nutrients, and therefore on the nutrient uptake capacity of the root system. Owing to the high K_{CO2} of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and the high N demand for construction of photosynthetic proteins, photosynthetic capacity is usually correlated to leaf N concentrations for a wide variety of natural or cultivated plant species (*e.g.*, Medina 1984, Field and Mooney 1986). Accordingly, nitrogen nutrition is fundamental for controlling leaf area construction (PAR interception) and PAR use by the canopy (photosynthetic capacity and transpirational cooling) for a given moisture and nutrients regime.

In general, very early secondary habitats are characterized by disturbances in soil N mineralization rates and fluctuations in total N content (Vitousek *et al.* 1989). Although N status and dynamics can be very variable, and obey to site-specific conditions, disturbing factors, such as fire and erosive processes in tropical forested ecosystems, may cause a temporary decrease in the total pools of soil available N by volatilization, leaching out or by decreasing organic matter mineralization rates. Two N nutrition strategies may be recognized among pioneer plants in such environments: (*a*) N acquisition through soil exploration and root system expansion; most plants rely on the efficiency of their roots for incorporation of soil inorganic nitrogen forms (NO_3^-; NH_4^+). (*b*) N acquisition through the fixation of molecular nitrogen from the atmosphere mediated by associations of roots with N_2-fixing microorganisms.

Photosynthetic carbon fixation has a high N requirement for the synthesis of RuBPCO and pigment-protein complexes in the chloroplasts. It is energetically more expensive to cover these requirements through symbiotic N_2-fixation than through the uptake of mineral N from soil (see Pate 1986). Therefore, we expect that different strategies of N acquisition and assimilation involve contrasting energetic costs that may be reflected in the saccharide balance of the leaves, and in N use efficiency in photosynthesis (amount of carbon fixed per unit leaf nitrogen). The relative importance of photochemical and biochemical steps of photosynthesis can be assessed using the relationships between chlorophyll (Chl) or N *versus* P_{max} in the field.

In this work we compared the P_{max} and P_{max}/N of two ecologically similar short-lived shrub species that rapidly colonize open spaces generated by logging and fire in montane cloud forest areas in northern Venezuela. *Crotalaria anagyroides* is a legume species that maintains N_2-fixing nodules under field conditions (Izaguirre Mayoral and Vivas 1996). *Verbesina turbacensis* is a composite species that relies on the availability of mineral forms of N in the soil for its N nutrition.

Materials and methods

Study site and plants: The study site is located in Altos de Pipe, Venezuela (10°20'N, 66°55'W), *ca.* 1500 m altitude. Annual rainfall is 994 mm, with precipitation predominantly occurring from May to December, followed by a short dry season from January to March (Cuenca 1986). Yearly average temperature is 17.6 °C. Predominant vegetation is a primary cloud forest, surrounded by secondary forests and grasslands. Seasonal fires leading to soil exposure are rather frequent during the dry season in the secondary vegetation units. Disturbed areas are dominated by annual herbs, grasses, and shrubs exhibiting rapid growth rates during the rainy season. For this study, individuals of *C. anagyroides* HBK (*Papilionoideae*) and *V. turbacensis* HBK (*Asteraceae*) growing together on a gentle slope, were chosen for comparative photosynthetic analyses. Both species establish and develop shortly after mechanical or combustive removal of vegetation, and reach sexual maturity and peak reproductive production by the end of the annual rain cycle.

Gas exchange measurements: The P_{max} of leaves of different age was measured for both plants growing in the field, using an open portable gas exchange system (*LCA-3*; *Analytical Development Co.*, Hoddesdon, U.K.). All the measurements were made between 09:00 to 12:00 h on clear sunny days from September to November. Ambient air [350 μmol(CO_2) mol^{-1}], drawn from a place far from any local CO_2 sources, was used as reference, and pumped through CO_2 impermeable plastic tubing to a Parkinson type leaf chamber (6.25 cm^2). For all the measurements, PAR was kept saturating [above 1.0 mmol(photon) m^{-2} s^{-1}]. Leaf chamber temperature was kept within 23-32 °C by using forced air convection (portable fan) and/or placing ice-watered towels over the leaf chamber aluminum structure when necessary. Day dark respiration rate (R_D) was measured by shading the leaf to total darkness in the field for 10 to 15 min, covering the chamber with aluminum foil. Calculations for the gas exchange physiological parameters were derived using the model of Farquhar *et al.* (1980). Immediately after gas exchange measurements, leaf discs of known area were punched out of some leaves, and stored either in dry ice (for Chl and saccharides) or in paper bags (for N), and transported to the laboratory for chemical analyses. When the amount of tissue of the measured leaf was too small for these analyses, several similar leaves were measured in parallel. In these cases the values of photosynthesis and composition were averaged.

N and Chl concentrations of leaves: For organic N determination, leaf discs of known fresh area were dried in a ventilated oven at 60 °C for at least 72 h. The material was weighed, ground, and digested in a sulfuric acid-*Kjeltabs*™ mixture at 350 °C for 2 h. Reduced nitrogen was distilled in a Kjeltech device, and recovered in a 2 % boric acid-pH indicators solution trap, which was later on titrated with 0.001 M HCl (micro-Kjeldahl). Nitrogen concentration was expressed per unit leaf dry mass or per unit leaf area after determining the fresh leaf area to dry mass ratio for each leaf sampled. Chl concentration was determined by visible range spectrophotometry of 80 % acetone leaf extracts from frozen leaf disks, following Arnon (1949).

Non-structural saccharide content: Field frozen disks from leaves used for gas exchange analysis were thawed and oven-dried at 60 °C until constant mass at the laboratory. The dry material was thereafter ground, and soluble saccharides extracted separately in boiling water and boiling 80 % ethanol. Insoluble saccharide fraction was extracted in 52 % perchloric acid from the same leaf pellets. The extracts were individually analyzed for saccharides using the anthrone method (Hassid and Neufeld 1964). The saccharide content was expressed as glucose equivalents' mass per unit leaf dry mass.

Results

Maximum photosynthetic rate, nitrogen content, and leaf structure: P_{max} increased linearly with leaf N content in both *Crotalaria* and *Verbesina*, expressed per unit leaf area or mass (Fig. 1*A,B*). In a wide variety of species such relationships are stronger on a leaf mass basis than on a leaf area basis (*e.g.*, Medina 1984, Field and Mooney 1986, Evans 1989, Ellsworth and Reich 1996). The slope of the relationship was higher in the N_2 fixer than in the non-fixer counterpart, both per unit leaf area and mass (Fig. 1*A,B*; Table 1). The regression showed a negative y-intercept for the N_2-fixer species while the y-intercept was not significantly different from zero in *Verbesina* when expressed per unit leaf mass (Fig. 1*B*; Table 1). Consequently, the instantaneous photosynthetic nitrogen use efficiency (IPNUE) increased with leaf N content in the N_2-fixer species in a curvilinear fashion (as expected from the relationship: $P_{max}/N = m + b/N$; where m is the slope, and b is the y-intercept of the P_{max}-N relationship, respectively), while it remained approximately constant (*i.e.*, $P_{max}/N = m$) in the non-fixer species (Fig. 1*C*).

Both species showed similar range of N content per unit leaf area (Table 2) but differed in the N content per unit leaf mass (Fig. 1*A,B*). This resulted from generally lower SLA in *Verbesina* leaves as compared to those of *Crotalaria* (Table 2). The nitrogen compensation concentration (NCC), defined as the leaf N concentration where net carbon gain equals zero (Table 1), was higher in *Crotalaria* than in *Verbesina* per unit leaf mass (Fig. 1*B*). No biologically meaningful NCC, however, could be derived for the per unit leaf area relationship in *Verbesina* (y intercept > 0; Table 1). These relationships showed that *Crotalaria* had higher P_{max} than *Verbesina* for all N concentrations above 60 mmol m^{-2} or 1.8 mol kg^{-1} (Fig. 1*A,B*).

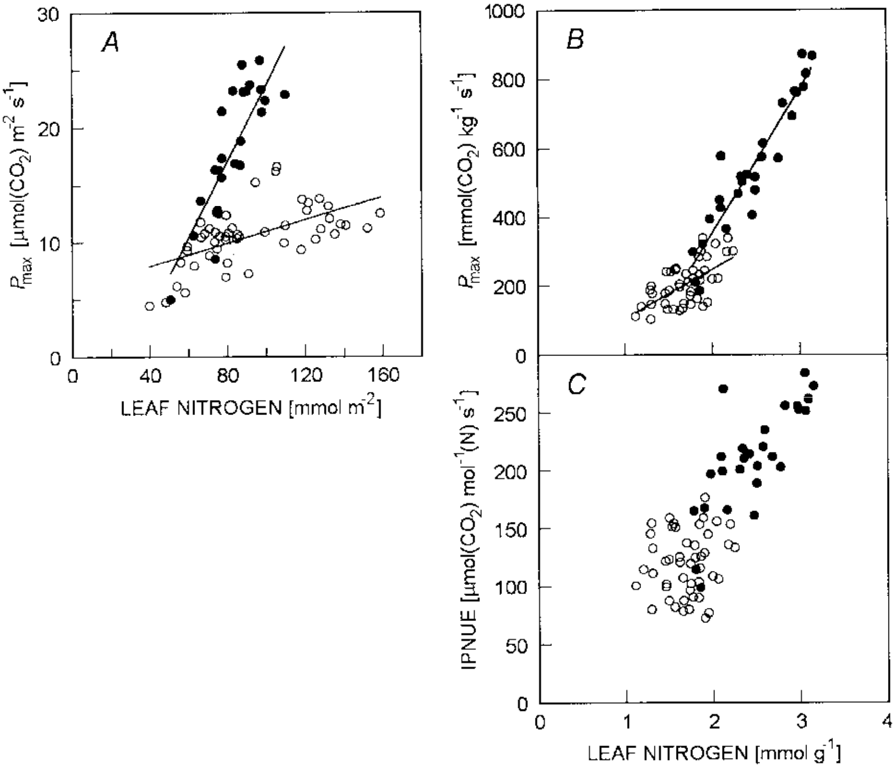

Fig. 1. Radiant energy saturated photosynthetic rate (P_{max}) as a function of age-related leaf nitrogen content per unit area (*A*) or per unit mass (*B*), and intrinsic photosynthetic N use efficiency, IPNUE for all the leaves studied (*C*). ●, *Crotalaria anagyroides*; ○, *Verbesina turbacensis*.

P_{max}, leaf conductance to water vapour (g_s), and intrinsic water use efficiency: The g_s was positively correlated with P_{max} in both species (Fig. 2) for the whole range of evaluated leaves differing in their age-related leaf N content. In general, intrinsic water use efficiency (IWUE), defined as P_{max} per unit g_s to water vapour, was lower in mature leaves of the N_2-fixer than in mature leaves of the non-fixer plant (Table 2). The c_i/c_a ratio of mature leaves in *Crotalaria* was higher than the mean c_i/c_a ratio of *Verbesina* mature leaves (Table 2).

N and Chl contents: The Chl content in leaves of both species was linearly related to N/area (Fig. 3*A*). The y-intercept for *Crotalaria* regression was near to zero (Fig. 3*A*; Table 1), and consequently the ratio Chl/N per unit leaf area tended to remain constant throughout the whole range of N concentrations in leaves. For *Verbesina* there was a slight, yet statistically significant, decrease in the Chl/N ratio with increasing leaf N/area as a consequence of the positive y-intercept derived from the linear regression of Chl over N/area (from Table 1). The slopes of the linear regressions for each species differed significantly. The slope of *Crotalaria* regression was less than two-fold higher than that of *Verbesina*, while the N/area *vs.* P_{max} regression showed a six-fold higher slope in *Crotalaria* than in *Verbesina* (Table 1).

The relationship between P_{max} and Chl content was not significantly different between the two species (Fig. 3*B*; Table 1), indicating that the observed decrease in carbon fixation capacity, related to age depending decreases in leaf N, is explained by the reduction of N compounds other than those associated with the light-harvesting chlorophyll-protein complexes.

Table 1. Regressions between radiant energy saturated photosynthetic rate (P_{max}), N content, chlorophyll content, and specific leaf area (SLA). For the N, gas exchange, and structural relationships, the sample size consisted of 31 *Crotalaria* leaves and 50 leaves from *Verbesina*. The Chl-N regressions were derived from 27 leaves of *Crotalaria* and 22 *Verbesina* leaves. NS indicates a non-significant relationship ($p>0.05$), and therefore no regression parameters are shown. Unless followed by a same letter, the paired comparisons between species are different for $p<0.05$.

Species	Independent variable	Dependent variable	r^2 ($p<$)	Slope	Intercept
Crotalaria	N/area	P_{max}/area	0.70 (0.01)	0.332	-9.71
Verbesina	N/area	P_{max}/area	0.35 (0.01)	0.051	5.79
Crotalaria	N/mass	P_{max}/mass	0.99 (0.01)	29.80	-482.50
Verbesina	N/mass	P_{max}/mass	0.38 (0.01)	10.23	-40.88
Crotalaria	N/mass	SLA	0.19 (0.05)	43.50	191.44
Verbesina	N/mass	SLA	NS	NS	
Crotalaria	P_{max}	g_s	0.54 (0.01)	0.095	0.120
Verbesina	P_{max}	g_s	0.62 (0.01)	0.033	0.030
Crotalaria	N/area	Chl ($a+b$)/area	0.54 (0.01)	4.03	0.072
Verbesina	N/area	Chl ($a+b$)/area	0.62 (0.01)	2.45	0.131
Crotalaria	Chl ($a+b$)/area	P_{max}/area	0.74 (0.01)	60.52 (a)	-6.30 (b)
Verbesina	Chl ($a+b$)/area	P_{max}/area	0.71 (0.01)	50.28 (a)	-4.88 (b)

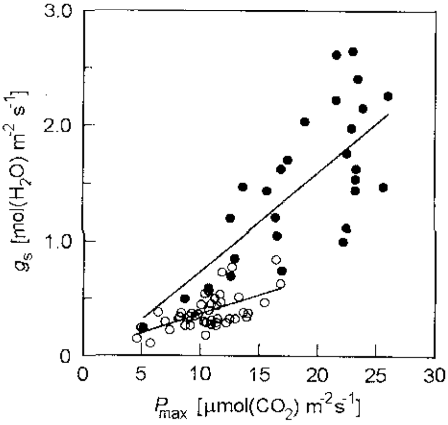

Fig. 2. Relationship between radiant energy saturated photosynthetic rate (P_{max}) and stomatal conductance to water vapour (g_s) under field conditions. ●, *Crotalaria anagyroides*; ○, *Verbesina turbacensis*.

Table 2. Structural and functional properties of the photosynthetic apparatus, and carbon balance parameters of mature leaves [specific leaf area (SLA), radiant energy saturated photosynthetic rate (P_{max}), stomatal conductance (g_s), chlorophyll (Chl) content, ratio of internal and external CO_2 concentrations (c_i/c_a), intrinsic water use efficiency (IWUE), respiration rate (R_D)] for *Crotalaria* and *Verbesina* evaluated in field. All differences among means are significant at $p<0.001$ (Student *t*-test).

Variable	*Crotalaria*	*n*	*Verbesina*	*n*
SLA [m^2 kg^{-1}]	29.20 ± 3.87	23	19.81 ± 4.68	33
Nitrogen content [mol kg^{-1}]	2.60 ± 0.35	23	1.79 ± 0.22	33
P_{max} [$\mu mol(CO_2)$ m^{-2} s^{-1}]	20.60 ± 3.90	23	11.60 ± 1.90	33
g_s [$mol(H_2O)$ m^{-2} s^{-1}]	1.65 ± 0.57	23	0.43 ± 0.14	33
c_i/c_a	0.94 ± 0.02	23	0.71 ± 0.04	33
IWUE [$\mu mol(CO_2)$ $mol^{-1}(H_2O)$]	13.70 ± 4.30	23	29.10 ± 7.30	33
Chl [mmol m^{-2}]	0.44 ± 0.06	23	0.32 ± 0.04	33
R_D [$g(CO_2)$ kg^{-1} s^{-1}]	4.30 ± 0.80	12	1.70 ± 0.40	8
R_D [$g(CO_2)$ m^{-2} s^{-1}]	2.90 ± 0.50	12	1.50 ± 0.20	8
Total non-struct. saccharides [g(glucose) kg^{-1}]	102.20 ± 27.60	20	133.00 ± 16.10	26
Soluble saccharides [%]	40.80 ± 5.80	20	26.30 ± 6.50	26

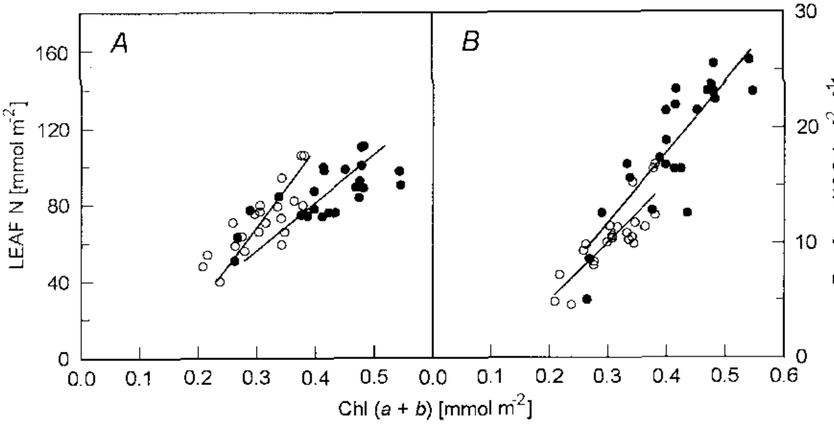

Fig. 3. Relationships between chlorophyll (*a*+*b*) concentration per unit area and (*A*) leaf organic nitrogen content per unit leaf area, or (*B*) radiant energy saturated photosynthetic rate (P_{max}) under field conditions. ●, *Crotalaria anagyroides*; ○, *Verbesina turbacensis*.

P_{max}, leaf total non-structural saccharides, and leaf respiration: A mean mature leaf of *C. anagyroides* showed higher P_{max} and concurrent lower total non-structural saccharides concentration (TNSS) per unit leaf mass than a mature leaf from *V. turbacensis* plants (Table 2). Under similar temperature ranges, mature leaves of *Crotalaria* subjected for a few minutes to total darkness showed higher R_D than mature leaves of *Verbesina* (Table 2). Whether these values are independent or not of photosynthetic CO_2 assimilation is not yet clear, and they are probably related to the pool size of post-irradiation photorespiratory metabolites.

Discussion

P_{max}, N content, and SLA in leaves of both species were similar to previously reported values for early successional species (*e.g.*, Bazzaz and Picket 1980, Ellsworth and Reich 1996). Both species showed similar range of N/area but the N_2-fixer species showed a greater N/mass than the non-fixer species. This resulted from lower SLA in leaves of the non-fixer. SLA is related to the relative growth rate through the net assimilation rate and the leaf mass/plant mass ratio, and hence whole plant carbon balance can be sensibly affected by this parameter (*e.g.*, Pearcy and Sims 1994). Although some clear relationships between SLA, P_{max}, and N have been found in rapid growing species (*e.g.*, Medina 1984), it is probably a very plastic plant trait that can be affected by genotypic and environmental factors in non-straightforward ways (Dijkstra 1989). SLA was rather loosely related to leaf N in *Crotalaria* while no relationship at all could be detected in *Verbesina* (relationships not shown). Additionally, leaves of *Verbesina* showed a larger range of SLA (10.9-32.0 m^2 kg^{-1}) than those of *Crotalaria* (24.0-36.0 m^2 kg^{-1}). This suggests that *Verbesina* may adjust SLA to changes in environmental conditions for water and nutrient availability in a more plastic manner than the N_2-fixer species.

The slope of the linear relationship P_{max}-N was higher for the N_2-fixer species than for the non-fixer indicating that P_{max} responds more strongly to increments in N in *Crotalaria* than *Verbesina* leaves. Moreover, the ratio c_i/c_a remained roughly constant throughout the range of N in *Crotalaria* leaves, indicating that differences in photosynthetic rate among leaves can be attributed to changes in biochemical capacity, and not to diffusion limitations of CO_2 through stomata. Despite the higher assimilation capacity of mature leaves in *Crotalaria*, they showed mean c_i values closer to the atmospheric CO_2 concentration in comparison to *Verbesina* mature leaves developed and measured under the same field conditions. This indicates greater stomatal limitations for CO_2 diffusion from the atmosphere into the intercellular spaces in mature leaves of the non-fixer as compared to the N_2-fixer. The g_s values in the N_2-fixer species were much higher than those of the non-fixer species leading to the observed lower IWUE. An inverse relationship between IPNUE and IWUE has been described for several species growing in natural or cultivated conditions (*e.g.*, Field and Mooney 1986, Sage and Pearcy 1987). It reflects the basic compromise between CO_2 and water vapour diffusion through stomata in relation to the biochemical capacity for photosynthetic carbon reduction. The lower IWUE in the studied N_2-fixer species is in agreement with results of Schulze *et al.* (1991) in an arid gradient in African savannas. In that study, N fixation (as revealed by $\delta^{15}N$) in *Mimosa* was inversely correlated to an aridity gradient and intrinsic water use efficiency (as revealed by $\delta^{13}C$).

Considering the similar Chl content-N relationships of the two species, it is probable that the remarkably higher IPNUE of *Crotalaria* leaves is explained by larger allocation of N to carbon reduction proteins than to photon harvesting proteins, as compared to *Verbesina*. The relative apparent decrease in IPNUE for high N/area leaves in *Verbesina* could result from N allocation to compounds not directly contributing to the observed P_{max} (cf. Field and Mooney 1986).

Despite their higher P_{max}, leaves of the N_2-fixer species showed lower TNSS content than leaves of the non-fixer counterpart. This points to larger saccharide sinks in the N_2-fixer, associated to energy expenses in N_2 fixation by the root nodules, and higher leaf R_D.

In summary, biochemical and structural adjustments for photosynthesis seem to be coupled to leaf N content in both species as it varies with leaf age. Both species can be distinguished by their different IPNUE and their IWUE at the leaf level. Nitrogen fixation capacity in *Crotalaria* is associated to higher IPNUE, lower water-use efficiency, and higher saccharide consumption by leaf respiration and root nodules activity. Nitrogen fixation capacity may confer competitive advantage in the occupation of nitrogen depleted environments, particularly in patches where PAR, water availability, and nutrients other than N are not limiting, and specific physicochemical requirements for symbiotic N_2 fixation are met (Pate 1986). Instead, reliance on N acquisition from soil, associated to relatively lower relative growth rates, may allow a greater plasticity in other plant functional traits, and thus larger ranges of space occupation in the spatio-temporal heterogeneous environment that characterize secondary habitats.

IPNUE is only one functional trait of plant carbon-nitrogen balance directly associated to shoot PAR use for carbon flux density at the leaves. Other components, such as root and shoot architecture, and allocation patterns in relation to exchange surfaces´ dynamics are required for better understanding the dynamics of space occupation in early secondary environments.

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