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Photosynthetic parameters of young Brazil nut (*Bertholletia excelsa* H. B.) plants subjected to fertilization in a degraded area in Central Amazonia

M.J. FERREIRA, J.F.C. GONÇALVES+, and J.B.S. FERRAZ

Instituto Nacional de Pesquisas da Amazônia (MCT-INPA), Coordenação de Pesquisas em Silvicultura Tropical, Caixa Postal 478, Manaus 69011-970, AM, Brazil

Abstract

In an experimental site for reforestation of degraded area, three-year-old plants of *Bertholletia excelsa* Humb. & Bonpl. were subjected to different fertilization treatments: T0 = unfertilized control, T1 = green fertilization (branches and leaves) and T2 = chemical fertilization. Higher net photosynthetic rates (P_N) were observed in T1 [13.2±1.0 μmol(CO_2) m^{-2} s^{-1}] compared to T2 [8.0±1.8 μmol(CO_2) m^{-2} s^{-1}] and T0 [4.8±1.3 μmol(CO_2) m^{-2} s^{-1}]. Stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE) of individuals of T1 and T2 did not differ significantly, however, they were by 88, 55 and 63%, respectively, higher in T1 than in the control. The mean values of variable fluorescence (F_v), performance index (P.I.) and total chlorophyll [Chl ($a+b$)] were higher in T1. Our results indicate that green fertilization improves photosynthetic structure and function in plants of *B. excelsa* in young phase.

Additional key words: chlorophyll *a* fluorescence; chloroplast pigment contents; forest restoration; green fertilization; performance index; photosynthesis.

Introduction

Plants growing in degraded areas are subjected to different stress levels in regard to water, light and nutrient supply, among other site factors, more than plants growing under more favorable conditions. The capacity to develop adaptative strategies could favor plant establishment and growth in degraded areas (Puerta 2002). This includes mechanisms related to photosynthetic efficiency, radiant energy utilization, carbon assimilation and regulation of water absorption, according to the needs of each species (dos Santos *et al.* 2006). One of the main abiotic changes that occurs with the removal of the original forest cover is a significant increase in irradiance level (Gonçalves and Santos Júnior 2005). Under high irradiance levels, plants can undergo changes in the contents of chloroplast pigments, short-term reduction in the activity of antioxidant enzymes, and also photoinhibition of photosynthetic reaction centers (Jiao *et al.* 2004, Morais *et al.* 2007).

Soil nutrient availability and foliar nutrient concentrations together with other abiotic factors such as high light intensity also constitutes a potential limiting factor for the optimal growth and development of plants. On the hand, an adequate nutrient supply can favour different growth mechanisms of plants such as photosynthetic performance, contributing specially for the establishment of plants in the field (Marenco *et al.* 2001, Silva *et al.* 2008). The nutrients in their multiple functions confer higher stability to the performance of vital plant processes. The positive relationship between N, chlorophyll (Chl) and photosynthesis is well known (Evans 1989, Fritschi and Ray 2007, Makoto and Koike 2007). Some micronurients, *e.g.* Cu, Mn, Zn, and B can increase biotic and abiotic stress tolerance (Kirkby and Römheld 2004). However, the form in which these nutrients are made available for plants can be decisive for their growth performance. Care is then needed when adjusting nutrient

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+Author for correspondence; e-mail: jfc@inpa.gov.br

Abbreviations: *Car* – carotenoids; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Chl (*a*+*b*) – total chlorophyll; E – transpiration rate; g_s – stomatal conductance; F_m – maximum fluorescence; ($F_v = F_m - F_0$) – variable fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; F_0 – initial fluorescence; P_N – net photosynthetic rate; P.I. – performance index; T0 – unfertilized control; T1 – green fertilization; T2 – chemical fertilization; WUE – water use efficiency.

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levels in degraded soils to support plant nutritional requirements.

The Brazil nut tree (*Bertholletia excelsa* H. B.) is a forest species of the Lecythidaceae family. This family includes 200 species, distributed primarily in the Neotropics, from southern Mexico to southern Brazil. The genus *Bertholletia* is monospecific, with the Brazil nut trees its only species. It is a widely distributed tree, best adapted to the non-flooded Amazonian forests of the Guianas, Venezuela, Colombia, Peru, Bolivia and Brazil (Mori 1992). *B. excelsa* can be used in large forestry plantations, even in areas degraded by pasture or mining activities (Souza *et al.* 2008). Testing the photosynthetic activity of this species to elucidate its physiological performance under different growing conditions can support the decision making in reforestation programs for forest plantations or environmental restoration in Amazonia.

The knowledge gap in the biology and ecophysiology of species and their adaptations to environmental stress has limited reforestation programs. The number of ecophysiological studies of Amazonian tree species is still very small, especially when considering the high complexity of their environments and the different biotic and abiotic factors affecting the species (Pezzopane *et al.* 2002). Therefore, the present study aims to investigate the effects of different fertilization treatments applied in a reforestation of *Bertholletia excelsa* on degraded soil, assessing its response in CO_2 and water vapour exchanges, Chl *a* fluorescence and changes in the content of chloroplast pigments. We tested the hypothesis that among different fertilization treatments in degraded areas, a form of fertilization combining physical and chemical benefits, rather than chemical benefits alone, would confer a higher functional stability to the photosynthetic activity in young plants of *B. excelsa*.

Materials and methods

Study site and planting: The study area is located in Manaus – AM (60° 01’ 07” W and 03° 05’ 08” S). The original vegetation was a “Non-Flooded Dense Forest” with *Micrandropsis scleroxylon*, *Eschweilera tessmannii*, *Eschweilera romeu-cardosoi* and *Minquartia guianensis* as dominant species. The local climate is classified as “Amw”, with a mean annual temperature of 26.7°C and precipitation of 2,186 mm (Fisch 1990). About 3-ha forest area was clearcut in 1980 and the soil was removed to a depth of 3 m. The terrain was bulldozed and compacted for construction, however, the area was abandoned for the following 24 years. In March 2004, with the aim of reforesting the site, the terrain was manually prepared with plantation holes (30 × 40 cm), in 1 × 1 m distance, to receive the Brazil nut seedlings. With no canopy present, the seedlings were exposed to full irradiance and grown in a bare soil. In December 2007, the plantation seedlings (mean height = 0.9 m and stem diameter 10 cm above the ground = 2.3 cm) were subjected to different fertilization treatments: green fertilization with branches and leaves from a nearby secondary forest was applied to 200 trees (9 kg/tree; T1). The same number of trees was chemically fertilized (T2) with 150 g of Fosmag® (4% N, 14% P, 7% K, 11.5% Ca, 2.7% Mg, 10.4% S, 0.07% B, 0.59% Zn, and 0.15% Cu) and 50 g of lime, incorporated around the trunks. Another 200 plants received no fertilization (control, T0).

CO_2 and water vapour exchanges were measured between 9:00 and 12:00 h, in 10 randomly selected trees of each treatment sixty days after fertilization. In each tree a fully expanded healthy leaf exposed to sun was selected. Net photosynthetic rate (P_N), dark respiration (R_D), transpiration rate (E) and stomatal conductance (g_s) in the adaxial leaf side were measured with a portable open CO_2- and water vapour exchanges system with infrared gas analyzer (*LI-6400, LI-COR,* Lincoln, USA), as described by dos Santos *et al.* (2006). Each measurement for the above parameters was determined for photosynthetic photon flux densities (PPFD) of 0 and 1,500 μmol m^{-2} s^{-1}, with the foliar chamber adjusted for a CO_2 concentration, temperature and H_2O vapour of 380±4 μmol mol^{-1}, 31±1°C, and 21±1 mmol mol^{-1}, respectively. Water use efficiency (WUE) was calculated as the ratio P_N/E.

Chl *a* fluorescence was assessed with a portable fluorimeter (*PEA, MK2-9600, Hansatech,* Norfolk, UK) on 10 completely expanded and healthy leaves in each treatment. The selected leaves were adapted to a dark period of 30 min, sufficient for the complete oxidation of the photosynthetic electron transport system. The adaxial leaf side was then exposed to a 5-s excitation pulse of high irradiance (3,000 μmol m^{-2} s^{-1}) with a wavelength of 650 nm. The measurements were done between 9:00 and 12:00 h (irradiance levels between 2000–2300 μmol m^{-2} s^{-1}), on the same trees.

Determination of the chloroplast pigments: After CO_2 and water vapour exchanges readings, the marked leaves were collected for Chl and carotenoids (Car) pigment analysis according to Hendry and Price (1993). These were performed with 0.1 g of fresh material ground in 10 ml of 80% acetone and magnesium carbonate ($MgCO_3$) and immediately afterwards 10 ml of 100% acetone was added. The suspension was filtered and absorbance was read at 663 nm (Chl *a*), 645 nm (Chl *b*) and 480 nm (Car) using a spectrophotometer (*Ultrospec 2100 pro UV/ visible, Amersham, Biosciences,* Cambridge, UK) (Lichtenthaler and Wellburn 1983, Gonçalves *et al*. 2005).

Experimental design and statistical analysis: The experimental design was completely randomized with three treatments (T0, T1 and T2) distributed randomly amongst the population of trees. The measurements were carried out with 10 replicates of each treatment. The results of each parameter were subjected to one-way analysis of variance (*ANOVA*). The means were compared using the *Tukey* test ($p \leq 0.05$), with the statistical program *STATISTICA 6.0* (*StatSoft Inc*., Tulsa, OK, USA).

Results and discussion

CO_2 and water vapour exchanges: The CO_2 and water vapour exchanges of the individuals of *B. excelsa* showed significant differences among the three fertilization treatments (Table 1). P_N values were much higher in T1 trees [13.2 μmol(CO_2) m^{-2} s^{-1}], which showed P_N almost three times higher than the control (T0) [4.8 μmol(CO_2) m^{-2} s^{-1}]. All CO_2 and water vapour exchanges parameters of T1 trees always presented higher values when compared to T0. However when compared to individuals of T2, no significant differences were observed in g_s, E and WUE. In contrast, T2 seedlings had lower carbon assimilation than those of T1. The mean value of P_N of T1 trees was higher than the highest value [9.4 μmol(CO_2) m^{-2} s^{-1}] measured at the same PPFD (1,500 μmol m^{-2} s^{-1}) measured in individuals of the same species in a 10-year-old mixed forest plantation established on a degraded area (J.F. de C. Gonçalves, unpublished). It seems that beneficial WUE in T1 plants might be caused by increased water availability. Possible explanation is that foliage cover used in T1 treatment formed a layer over soil surface preventing soil evaporation and thus increasing water availability for root system of the T1 plants. Maybe a better soil microbiology properties might be expected from such arrangement. However, such mechanisms were not probably present in T2 plants. Such explanations might be also supported by the data presented in Table 1. E and g_s were insignificantly higher in T1 compared to T2 plants. Since WUE was found higher in T1 than in T2 plants one may hypothesise that water availability was higher in T1 than in T2 plants.

Chl fluorescence: The maximum photochemical efficiency (F_v/F_m) of *B. excelsa* in treatment T1 did not differ significantly from that in T2 plants. Analyzing the ratio F_v/F_m of T0 and T1 plants, the latter presented values that indicated lower stress than the control plants. These results may reflect better performance of primary photosynthetic processes in T1 and T2 plants (Table 2). As this experiment aims also to restore degraded areas through reforestation with plants being subjected to growth under stressing environmental conditions, it is necessary to understand the physiological bases of the strategies that are essential to their establishment. In this scenario, a more detailed analysis of Chl *a* fluorescence represents a tool for monitoring the effects of stress resulting from the high irradiance to which plants are subjected in open areas undergoing restoration processes (Gonçalves and Santos Júnior 2005). The performance index (P.I.) is a more appropriate parameter for the evaluation of the photosystem II (PSII) responses to stress conditions than the ratio F_v/F_m alone (Srivastava *et al*. 1999, Gonçalves *et al*. 2007). P.I. is a wider parameter because it is derived from the combination of structural and functional events of PSII that are responsible for electron transport during the photochemical phase of photosynthesis, and also refers to the energy that is dissipated or lost during this transport (Strasser *et al.* 1999, Contran *et al*. 2009). The values of F_v/F_m found in this study were similar to the values of adult *B. excelsa* trees in a 10-year-old mixed plantation (Morais *et al.* 2007).

Chloroplast pigment: The higher P.I. values and also the higher P_N observed in T1 plants can also be related to the higher contents of chloroplast pigments, specially Chl *a*,

Table 1. CO_2 and water vapour exchanges parameters and photosynthetical characteristics of plants of *Bertholletia excelsa* in area with degraded soil under different fertilizations. Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and water use efficiency (WUE). T0 – control, T1 – fertilization with fresh plant material (branches and leaves) and T2 – chemical fertilization. Mean of ten plants (±SD); mean values followed by the same letters between the treatments did not differ at $p \leq 0.05$ by the *Tukey* test.

Parameter	T0	T1	T2
P_N [μmol m^{-2} s^{-1}]	4.8 ± 1.3^c	13.2 ± 1.0^a	8.0 ± 1.8^b
g_s [mmol m^{-2} s^{-1}]	124 ± 61^b	233 ± 36^a	175 ± 84^{ab}
E [mmol m^{-2} s^{-1}]	2.7 ± 1.0^b	4.2 ± 0.4^a	3.4 ± 1.2^{ab}
WUE [μmol $mmol^{-1}$]	1.9 ± 0.5^b	3.1 ± 0.3^a	2.6 ± 0.8^a

Table 2. Chlorophyll *a* fluorescence parameters in plants of *Bertholletia excelsa* in an area with degraded soil under different fertilizations. Initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence ($F_v = F_m - F_0$), maximum photochemical efficiency (F_v/F_m) and performance index (P.I.). T0 – control, T1 – fertilization with fresh plant material (branches and leaves) and T2 – chemical fertilization. Means of ten plants (±SD); mean values followed by the same letters between the treatments did not differ at $p \leq 0.05$ by the *Tukey* test.

Parameter	T0	T1	T2
F_0	243 ± 28^a	228 ± 25^a	229 ± 79^a
F_m	712 ± 171^b	958 ± 177^a	752 ± 210^{ab}
F_v	468 ± 171^b	730 ± 180^a	522 ± 205^b
F_v/F_m	0.64 ± 0.09^b	0.75 ± 0.06^a	0.68 ± 0.11^{ab}
P.I.	0.16 ± 0.18^b	0.81 ± 0.6^a	0.36 ± 0.3^b

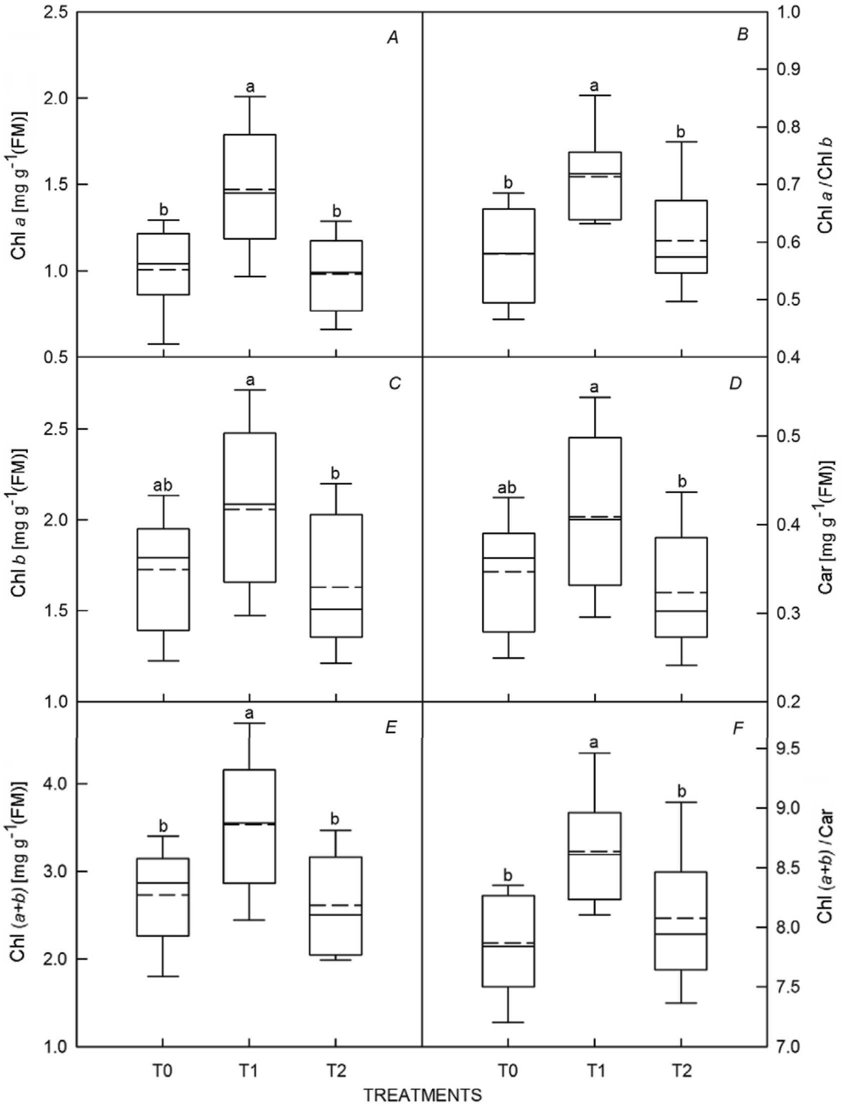

Fig. 1. Box plot of chloroplast pigment concentrations on a leaf fresh mass basis, for the different fertilization treatments (T0 – control, T1 – fertilization with fresh plant material (branches and leaves) and T2 – chemical fertilization). Chlorophyll *a* (*A*), chlorophyll *a*/*b* (*B*), chlorophyll *b* (*C*), carotenoids (*D*), chlorophyll (*a*+*b*) (*E*) and chlorophyll (*a*+*b*)/carotenoids (*F*). Box-plot area – 50% of the data, upper and lower bars – 25% of data variation, *continuous line* inside the box – median of data distribution, *dashed line* inside the box – mean value of data distribution. Mean values followed by the same letter between the treatments did not differ at $p \leq 0.05$ by the *Tukey* test ($n = 10$).

Table 3. Mean (± SD) concentration of macro and micro-nutrients in fresh plant material (branches and leaves - 9 kg/tree) used in fertilization at treatment T1 ($n = 4$).

Parameter	
C [g kg^{-1}]	514.0 ± 22.0
N [g kg^{-1}]	8.7 ± 1.6
P [g kg^{-1}]	0.3 ± 0.1
K [g kg^{-1}]	1.4 ± 0.3
Ca [g kg^{-1}]	4.3 ± 0.3
Mg [g kg^{-1}]	0.5 ± 0.2
S [g kg^{-1}]	0.8 ± 0.2
B [mg kg^{-1}]	5.3 ± 2.1
Cu [mg kg^{-1}]	4.8 ± 1.0
Fe [mg kg^{-1}]	108.0 ± 46.6
Mn [mg kg^{-1}]	75.3 ± 20.2
Zn [mg kg^{-1}]	14.7 ± 2.6

determined in these plants. The individuals in treatment T1 presented higher values for most of the pigments parameters (Fig. 1). Only Chl *b* and Car contents did not differ significantly between treatments T1 and T0. The higher Chl *a* content observed in individuals of T1 can be related to the higher N supply promoted by the fertilization with fresh plant material (branches and leaves) (Table 3). There is a correlation between the N status of the plant and the concentration of foliar Chl and consequently with a photosynthesis (Evans 1989, Reich *et al.* 1994). Most of the parameters of chloroplast pigments obtained in this study presented lower concentrations than those found in leaves of adult *B. excelsa* growing in agroforestry systems, with the exception of the parameter Chl (*a*+*b*)/Car (Morais *et al.* 2007). These results could reflect a protection strategy of the species *B. excelsa* to the stress caused by the high irradiance in the open area plantation in this study. Our results reflect three main conclusions: (*1*) the better establishment of the young *B. excelsa* trees, reflected by the better photosynthetic performance, as well as the better efficiency in the utilization of energy and accumulation of chloroplast pigments in T1 versus the control indicate it is possible to improve the seedling establishment, (*2*) the indistinguishable results between the chemical fertilizer treatment and the control indicate that adding nutrients alone is insufficient to improve seedlings success, and (*3*), finally, this study confirms the hypothesis that the improvement of the physical and chemical soil parameters obtained *via* fertilization with fresh plant material improved the photosynthetical process of *B. excelsa* and resulted in a better performance of the young trees on a degraded area.

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