

# Effect of irradiance on biomass growth and allocation in *Bertholletia excelsa* seedlings after enrichment planting



M.Sc. Thesis

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SUSTAIN 

# Effect of irradiance on biomass growth and allocation in *Bertholletia excelsa* seedlings after enrichment planting

M.Sc. Thesis by Jiska Schaaf, M.Sc. student Forest and Nature Conservation

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## **Abstract**

The economically and ecologically important Brazil nut tree (*Bertholletia excelsa*) is suffering from deforestation and habitat degradation, which could be counteracted by enrichment planting, i.e. increasing tree density in the forest. Therefore, knowledge about the best environmental conditions for enrichment planting of *B. excelsa* is important. In this study, the effect of irradiance on biomass growth and allocation (the relative growth rate (RGR), specific leaf area (SLA), leaf mass ratio (LMR), leaf area ratio (LAR), root-shoot ratio (R/S ratio) and leaf thickness) was investigated for *B. excelsa* seedlings planted in an enrichment program in the Madre de Dios region in the Peruvian Amazon. Furthermore, seedlings from nurseries and plantations were compared with each other to investigate differences in biomass allocation. The effect of irradiance on growth and biomass allocation was investigated with mixed effect models (MEMs) and a Structural Equation Model (SEM), whereas differences in biomass allocation amongst different planted seedling groups were analysed with ANOVA. This thesis has yielded two important results. MEMs showed that light had a significant positive influence on RGR, LMR and leaf thickness and a significant negative influence on SLA. Second, comparison of biomass allocation showed significant differences between the seedlings from the nursery and the seedlings from the fields. Based on this results, light conditions are crucial for enrichment planting success. Outplanting performance can be improved by better preparing the seedlings for transplantation through optimizing biomass allocation and length and by planting seedlings at optimal light conditions.

**Keywords:** forest conservation and management, *Bertholletia excelsa*, Brazil nut tree, non-timber forest product, enrichment planting, biomass allocation, growth responses, light, mixed modelling, SEM, outplanting performance, transplantation

## Introduction

### ***Protect ecosystems with NTFPs***

Due to a rapid increase of human demands, current rates of habitat degradation and deforestation endanger the future of forest resources and ecosystems (Cardinale *et al.*, 2012). Primary forests are decreasing and regenerating forests have become the predominant form of forest cover in the tropics worldwide (Chazdon, 2014). To increase attention to the importance of ecological life for society and to protect the forests, the economic valuation of ecosystem services is developed (Fisher *et al.*, 2009). The biggest value of the forest is attributed to timber, however non-timber forest products (NTFPs) can be worth a lot as well (Belcher & Vantomme, 2003; Peres *et al.*, 2003).

One way to increase the value of regenerating forests is through active restoration of NTFPs by enrichment planting, i.e. increasing tree density in the forest, with for example *Hevea brasiliensis* (rubber), *Spondias mombin* (nut) and *Manilkara spp.* (fruit) (Zuidema, 2003; Kainer *et al.*, 1998; Holl & Aide, 2011; Gunter *et al.*, 2011). Brazil nuts are in this context one of the most important NTFPs as they are the only internationally traded seed collected almost exclusively from natural (regenerating) forests (Peres *et al.*, 2003; Chunhieng *et al.*, 2004; Peña-Claros *et al.*, 2002; Zuidema, 2003). In 2016, Brazil, Bolivia and Peru exported US\$205 million worth of Brazil nuts (Food & Agriculture Organisation, 2018), therefore the seeds are an important income source for Brazil nut collectors (castañeros) in rural areas in the Amazon (Moraes *et al.*, 1994; Peres *et al.*, 2003). However, future production of *Bertholletia excelsa* seeds is threatened by deforestation, habitat degradation and reduced pollination services (Peres *et al.*, 2003). Variation in fruit and seed production can partly be attributed to changes in annual rainfall and drought patterns or seasonal temperature variables (Kainer *et al.*, 2007). Additionally, *B. excelsa* can face a lack of recruitment because of overharvesting or associated agouti hunting, one of the few animals that can open the Brazil nut fruits and main disperser of the seeds (Peres *et al.*, 2003). Consequently, population size cannot be maintained over the long term (Peres *et al.*, 2003). Protecting the Brazil nut production offers an opportunity to conserve nature and simultaneous to improve the livelihoods of castañeros (SUSTAIN, n.d.; Pierce *et al.*, 2003).

### ***Plant adaptation to light conditions***

In enrichment planting, seedlings can face problems after transplantation when water and sunlight availability are not similar at the new planting conditions compared with the nursery (Grossnickle, 2012). Plants respond to environmental conditions through changes in biomass growth and allocation to different plant parts (fig 1 and textbox 1) (Lambers *et al.*, 2008; Poorter & Nagel, 2000; Tyree *et al.*, 1998; Canham *et al.*, 1996). One of the most important requirements for plants, determining both the growth rate and the biomass allocation, is light. Light gives energy to the plant to grow through the process photosynthesis and gives informational signals that control the growth and development of the different parts of the plant to maximize the energy uptake (text box 1) (Lambers *et al.*, 2008). Plants in the shade invest in leaf growth to maximize the collection of sunlight, while plants in the sun invest in root growth for water uptake, to compensate for higher transpiration losses (Lambers *et al.*, 2008; Tyree *et al.*, 1998; Canham *et al.*, 1996; Poorter *et al.*, 2012). A growth analysis of plants of the same species growing in different light conditions can provide insight into those plant responses (Lambers *et al.*, 2008; pandey *et al.*, 2017). Biomass allocation can be investigated by calculating the specific leaf area (SLA), leaf mass ratio (LMR) or leaf area ratio (LAR) (fig 1) or by calculating the root-shoot ratio (R/S ratio), which is the ratio of the total dry mass in the roots to the total dry mass in the leaves of a plant (see fig 1 and text box 1 for further explanation) (Poorter & Nagel, 2000). Relative growth rate (RGR) is determined as the rate of increase in plant mass (Lambers *et al.*, 2008; Tyree *et al.*, 1998).

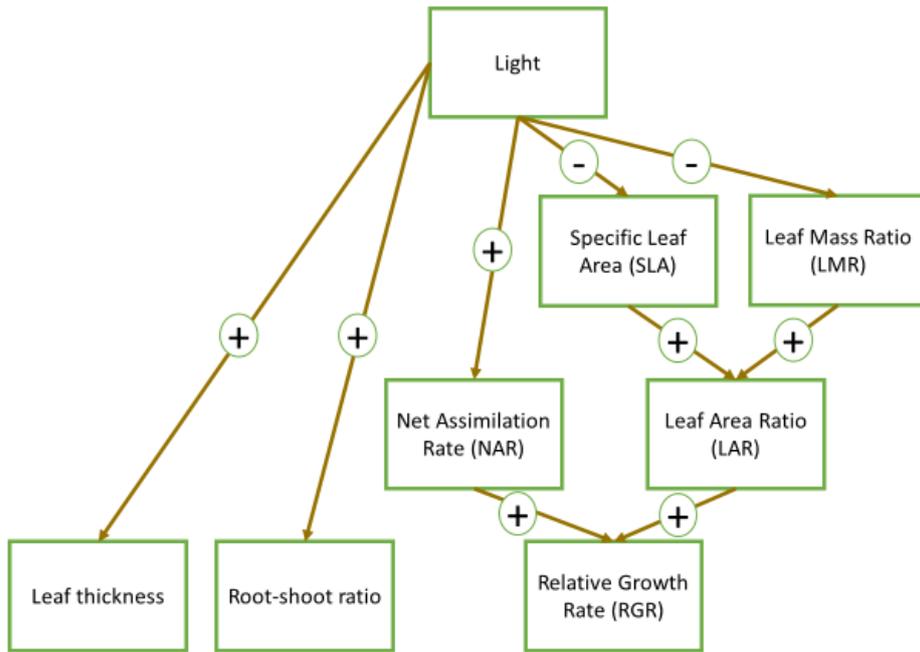


Figure 1. Conceptual framework showing the hypothesized effect of light on RGR and its components, leaf thickness and R/S ratio. The plusses and minuses indicate a positive or negative effect.

Information box. How to calculate LAR, LMR, SLA, RGR and NAR.

Abbreviation	Explanation	Unit
RGR	Relative growth rate	$\text{g g}^{-1} \text{ year}^{-1}$
SLA	Specific leaf area	$\text{cm}^2 \text{ g}^{-1}$
LMR	Leaf mass ratio	$\text{g g}^{-1}$
LAR	Leaf area ratio	$\text{cm}^2 \text{ g}^{-1}$
NAR	Net assimilation rate	$\text{g cm}^{-2} \text{ year}^{-1}$

$$\text{RGR} = \text{LAR} * \text{NAR} \quad (\text{formula 1})$$

LAR is the amount of leaf area per unit total plant mass. LAR consists of two components: SLA and LMR. SLA is the amount of leaf area per unit leaf mass. LMR is the fraction of the total plant biomass allocated to the leaves.

$$\text{LAR} = \text{SLA} * \text{LMR} \quad (\text{formula 2})$$

NAR is the rate of increase in plant dry mass per unit leaf area. For calculating the RGR (gram/year), the increase in dry plant mass ( $M_{P2} - M_{P1}$ ) needs to be calculated by successive weighting of different plants.

$$\text{RGR} = \frac{(\ln M_{P2} - \ln M_{P1})}{(t_2 - t_1)} \quad (\text{formula 3})$$

The RGR can be used to calculate an estimate of the mean NAR for the time-interval ( $t_2 - t_1$ ):

$$\text{NAR} = \frac{\text{Ln}(dMP)}{dt} / \left( \frac{A_L}{M_L} \times \frac{M_L}{M_P} \right) \quad (\text{formula 4})$$

$M_P$  is the total dry plant mass,  $A_L$  is the leaf area,  $M_L$  is the dry leaf mass

Text box 1. Calculation of RGR, LAR, SLA, LMR and NAR from Lambers et al., 2008 and Vernon & Allison, 1963.

### ***Aims and hypotheses***

Currently, enrichment planting of Brazil nut trees has been carried out at relatively small scale and has barely been monitored, hence knowledge about success remains limited. If measured, irradiance is often scaled in small light ranges, lines or gaps and not measured at maximal light conditions (Zuidema, 2003; Peña-Claros *et al.*, 2002; Kainer *et al.*, 2007; Kainer *et al.*, 1998; Myers *et al.*, 2000). In this study, light data was measured in a quantitative index of shading and carried out in collaboration with a large-scale enrichment planting program in the Madre de Dios region in Peru, aiming to determine growth and biomass allocation responses of *B. excelsa* seedlings to irradiance after enrichment planting. Furthermore, investigated are the differences in biomass allocation of *B. excelsa* seedlings in the nursery to those in the plantations as indication of the preparation of the seedlings to transplantation. Knowledge on management practices contributes to improve enrichment planting schemes and can therefore enhance population sizes of *B. excelsa* in the long term, improving the livelihoods of the castañeros and conserving natural ecosystems.

Subsequently,

1. It was hypothesized that light had a positive effect on the
  - RGR of *B. excelsa* seedlings because of an increase in the photosynthetic rate
  - R/S rate because of an increase in the biomass to the roots to compensate for the water loss by higher water uptake and a decrease in the leaf biomass to minimize the water loss by evaporation
  - leaf thickness because of an increase in the cell layers and chlorophyll to protect against irradiance

and a negative effect on the

- SLA because of a decrease in surface area of the leaf under higher light conditions and because the increase in leaf thickness causes an increase of leaf mass per leaf area
  - LMR because of a decreased leaf growth
  - LAR because LAR is defined by LMR and SLA which are both hypothesized to be negatively influenced by light
2. It was hypothesized that when *B. excelsa* seedlings in the nursery were not well prepared to the environmental conditions in the fields, their biomass allocation would not be similar to the biomass allocation of seedlings from the fields.

## Materials and Methods

### *Study Species: The Brazil nut tree*

*Bertholletia excelsa* is a long-lived light demanding pioneer species from the family Lecythidaceae, growing mainly in the lowlands of the Brazilian, Peruvian and Bolivian Amazon (Peña-Claros *et al.*, 2002). *B. excelsa* grows in non-flooding areas with rainfall ranging from 1450 to 3400 mm/year and a dry season of 2-7 months (Moraes *et al.*, 1994). *B. excelsa*, also called the ‘cornerstone of Amazonian conservation’ shows a high contribution to the aboveground carbon storage in forests of southwestern Amazonia (Guariguata *et al.*, 2017; Galia Selaya *et al.*, 2017). The tree may attain heights of up to 50 meters and a circumference of over 9 meters (Zuidema, 2003). The ball-shaped fruits are filled with 10 to 25 Brazil nuts (Peres *et al.*, 2003). These valuable seeds are harvested and traded, or the oil is extracted and used in a wide variety of products, mostly in the USA, Asia and Europe (Peres *et al.*, 2003; Chunhieng *et al.*, 2004). The tree relies on large-bodied bees for pollination and gene flow (Zuidema, 2003). *B. excelsa* is classified in the IUCN red list as vulnerable and is illegal to fell due to its ecological and socioeconomic value (Wildscreen Arkive, n.d.).

### *Study Area*

The Madre de Dios region (12°53'S 69°10'W) in southeast Peru contains more than 2.6 million ha of the most biologically diverse, carbon dense Brazil nut-rich forest (Tambopata National Reserve, 2019). Mean annual temperature is 25 °C, mean annual precipitation 2221 mm/year and the wet season lasts from December to March (Climate-data, 2015). The climate in the study area is classified as tropical savanna climate, Aw, the vegetation as lowland Amazonian rainforest consisting mainly of disturbed areas differing in successional stage (Tambopata National Reserve, 2019). In the study area, different enrichment planting sites were established between March and June 2018 by a local foundation and organisation North of Puerto Maldonado around the villages Alegria and Planchon (appendix 1) in cooperation with local concessionaires as beneficiaries.

### *Involved projects, foundations and organisations*

This study was done in collaboration with SUSTAIN, Fondo de Promoción de las Áreas Naturales Protegidas del Perú (PROFONANPE) and Instituto de Investigaciones de la Amazonía Peruana (IIAP). SUSTAIN is a research project of the university ETH Zurich and CIFOR that aims to identify the ecological, social and economic sustainability of the Brazil nut supply chain from Madre de Dios to Switzerland (SUSTAIN, n.d.). SUSTAIN arranged the collaboration with PROFONANPE and IIAP. IIAP contributes to research on conservation of natural resources of the Peruvian Amazon and owns an experimental station with nurseries close to the city of Puerto Maldonado where Brazil nut seedlings are germinated all year round. PROFONANPE is a private environmental foundation contributing to the conservation of biodiversity in Peru. One of the projects of PROFONANPE is enrichment planting of *B. excelsa*. The seedlings are first raised in the nurseries from IIAP, bought by PROFONANPE and given to preselected castañeros. Part of these were the seedlings used in this study.

### *Study design*

Data from 14 different enrichment planting fields in the Madre de Dios region in Peru was collected between September and November 2018. The fields were enriched with 50 or 100 *B. excelsa* seedlings, planted at least 10 m apart and 30-50 cm into the soil, with nets as individual fences to protect against herbivory (F. Chiriboga Arroyo, pers. comm, April 20, 2018). Light intensity was measured for 5 random seedlings per field before removing them for growth analysis. Site age, defined as the time since planting, ranged from 3 to 8 months with an average of 5.6 months (appendix 2).

### *Field measurements*

A commitment document (Acta de compromiso, appendix 3) was created in which on behalf of SUSTAIN 3 seedlings per harvested seedling was promised to compensate the castañeros. This document was signed by Fidel Chiriboga, the castañero, me and my field assistant. I always carried with me an explanation of my study written in Spanish (appendix 4).

A random number table was used to select 5 random seedlings (appendix 5). With the Lieberman method, light intensity was quantified by measuring the amount of shade per seedling, using the surrounding trees (Lieberman *et al.*, 1989). Distances and heights were measured using a Nikon laser ‘Forestry Pro’ (Specification: waterproof 6x21 6) or a tapeline. The Lieberman method calculates the angle ( $\alpha$ ) using the  $\sin^{-1}$  of the height of a tree ( $w$ ) divided by the distance from top seedling to top tree ( $y$ ) (appendix 6). The value of the light condition ( $G$ ) is calculated using the formula:  $G = \sum \sin(\alpha_1 + \alpha_2 + \dots + \alpha_n)$ . Every tree that influences the light intensity of the *B. excelsa* seedling was included. Outcomes ranged from 0 (full light) to 12 (little light) (appendix 7). A disadvantage of the Lieberman method is that it does not consider the size of the branches and leaves of the tree. This might have led to an under- or overestimation of the parameter value. In my study, fields were divergent in light conditions, therefore the Lieberman method was a suitable method to measure light intensity.

To calculate the RGR, changes in total dry weight between seedlings from the fields and seedlings from the nursery were calculated. The average of 10 seedlings from the nursery with an age of 4 months was measured (time=1). Time=2 were the measurements of the field seedlings.

### *Laboratory work*

In the experimental station of IIAP, leaf thickness was measured at 4 random points per leaf using a micrometer. Leaf area was calculated using pictures with the program ImageJ (Abràmoff *et al.*, 2004). Seedlings were dried, separated in paper bags, for

48 hours at 70 °C. Thereafter, the dry biomass of all different plant parts; the roots, nut, stem and leaves were weighted. Based on the leaf area and dry mass of the different plant parts the RGR, SLA, LMR, LAR and R/S ratio were calculated.

### **Data analysis**

A total of 70 data points was available for statistical analyses (5 seedlings x 14 fields). An ANOVA test was performed with the `aov` command in R studio to investigate the differences in light levels between the 14 fields (Chambers *et al.*, 1992), followed by a post hoc test with the `pairwise.t.test` command. Important to keep in mind is that light was measured with the Lieberman method, where a high G means low light and a low G high light.

### **Statistical analysis**

#### **Hypothesis 1 part 1**

To investigate the influence of different light conditions on the biomass growth and allocation of planted *B. excelsa* seedlings, mixed effect models (MEMs) were used, carried out using R studio version 3.5.2. RGR, SLA, LMR, LAR, R/S ratio and leaf thickness were the test variables. 'light' (G) and 'time spent in the field' (time) were the fixed effects. 'Biomass present when harvested' (biomass) was considered as a fixed effect only for leaf thickness because biomass was used in the calculation of the other test variables. Fields was the random effect. All fixed effects were standardized with the scale function in R studio (Eager, 2017). An interaction effect between light-time was included. For leaf thickness, the starting model included also the interaction between time-G and biomass-G.

MEMs were made in R studio using the `lme4` package (Bates *et al.*, 2014). A starting model including all explanatory variables, interaction terms, slope and intercept effects was constructed (appendix 10). Backwards selection was applied manually using the Akaike Information Criteria (AIC). If the AIC values of two models differed less than 4 points after dropping a variable, the most complete model was selected. It should be noted that sometimes the AIC was very similar for G or time as explaining variables, but not for both in the same model. First all models were fitted using Maximum Likelihood estimation (ML), the final model was refitted using Restricted Maximum Likelihood (REML). Models were validated by the visual inspection of the distribution of the residuals vs the fitted values of the model and a Shapiro-Wilk normality test was done to test for normality. SLA and R/S ratio were log transformed. For the final model was investigated which fixed effect was having a significant ( $p < 0.05$ ) effect on the test variable. The visualisation of the predicted relationship between the fixed and random effects and the test variable was made with the `ggplot` package (Wickham, 2016). The marginal and conditional  $R^2$  values were calculated with the `MuMIn` package (Nakagawa & Schielzeth, 2013).

#### **Hypothesis 1 part 2**

The conceptual diagram shows that the variables of the MEMs are interconnected, which influences the size of the coefficients found. In a structural equation model (SEM) the significance and strength of a relationship can be analysed in the context of the complete model. The standardized path coefficients indicate the direct effect of a variable on another variable while holding the other relations constant (implemented in the R package `semPlot` [Epskamp, 2015]; Dion, 2008). Path coefficients are based on series of regressions. In this model, 4 series of regressions were made:  $SLA \sim G$ ,  $LMR \sim G$ ,  $LAR \sim SLA + LMR$  and  $RGR \sim LAR + G$ . SEMs also test the overall correctness of the whole path model. Given the relatively small sample size (70), the robust Chi-square statistic is less reliable and the model-fit index Comparative Fit Index (CFI) was used in addition as it is less sensitive to sample sizes (Schermelleh-Engel *et al.*, 2003). For the model fit, the robust Chi-square should be  $> 0.05$  and CFI  $> 0.9$ , which was the case. The robust Chi-square is a correction of the Chi-square value in SEM by the nested data, which was needed to obtain a non-significant p-value (implemented in the R package `lavaan.survey` [Oberski, 2014]) (Savalei, 2014). Other widely applied model-fit indexes like RMSEA and the TLI were also tested, however those did not indicate an excellent model fit.

#### **Hypothesis 2**

I compared SLA, LMR, LAR, R/S ratio, leaf thickness and dry weight of the cotyledon between 4 groups of seedlings; one with 4 months-old seedlings growing in the nurseries (10 individuals, time=0) and the second, third and fourth group with seedlings that were planted in the field since on average 4, 5.5 and 7 months. Differences amongst groups were tested with ANOVA if variances were homogeneous, or Kruskal-Wallis tests as a non-parametric alternative (SPSS Statistics Version 25). Tukey HSD and Dunn-Bonferroni post hoc tests were thereafter respectively used to see where the differences laid.

## Results

Light conditions differed significantly among fields ( $F(2,13)=38.41$ ,  $p<0.05$ ), but were rather homogeneous within most fields (appendix 7 and 8). Dry weight of plant parts differed quite strongly among seedlings. Dry weight tended to be higher for plants in high light and for older plants (fig 2).

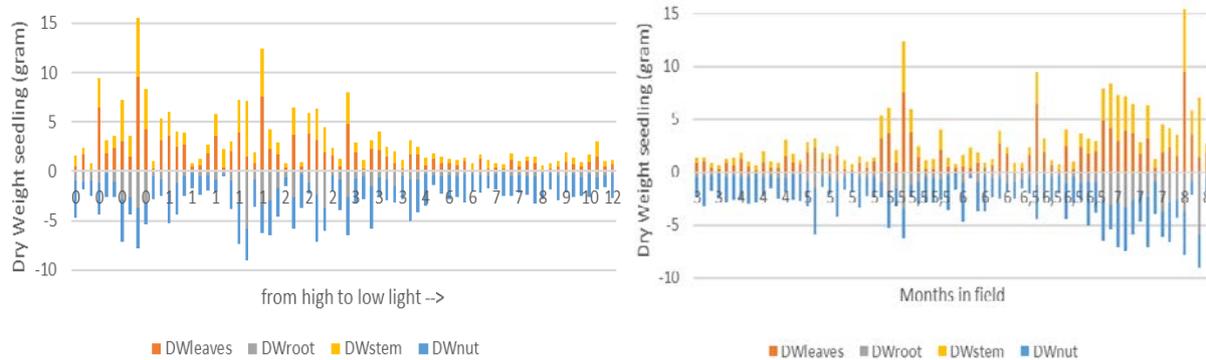


Figure 2. Dry weight of the 70 seedlings divided into aboveground and belowground parts, plotted against the light conditions (left) and the months the seedlings grew in the field (right). DW stands for dry weight.

### Hypothesis 1 part 1: Biomass growth and allocation

Final mixed effect models (MEMs) and the output of all the significant explaining variables of the final models are presented in table 1 (see appendix 9 and 11 for correlations between explanatory variables and results from Shapiro-Wilk normality tests respectively). Table 2 contains the explained variances for the random (variance by fields), fixed effects (marginal variance), residuals (variance by residuals), and the final MEM (conditional variance).

Table 1. Results of MEMs, coefficients and p-values for the relative growth rate (RGR), the LOG of specific leaf area (SLA), leaf mass ratio (LMR), leaf area ratio (LAR), the LOG of root/shoot ratio (R/S ratio) and leaf thickness. Significant effects ( $p<0.05$ ) are coloured in black and non-significant effects are coloured in grey. Blank cell means a variable was not included in the model. The explaining variables are standardized.

Test variable	Final model	G	Time	Biomass	Interaction effect	Intercept	Random slope for G
RGR	G+time+G*time	<b>-0.43</b> (p=0.002)	<b>0.22</b>		<b>G*time</b> 0.28 (p=0.041)	0.5	Yes
Log SLA	G	<b>0.08</b> (p=0.008)				4.99	No
LMR	G	<b>-0.04</b> (p=0.027)				0.23	Yes
LAR	G+time+G*time	<b>-4.25</b>	<b>-0.34</b>		1.6	32.17	Yes
Log R/S ratio	G+time+G*time	<b>0.07</b>	<b>0.08</b>		0.07	1.09	Yes
Leaf thickness	Biomass+G* biomass+G*time	<b>0</b>	<b>0</b>	<b>0.02</b> (p=0.000)	<b>-0.01</b> <b>G*time</b> (p=0.009) <b>0.02</b> <b>G*biomass</b> (p=0.000)	0.19	Yes

Table 2. Variance explained by the random effect fields and variance explained by the residuals of the model. The  $R^2$  of the model is categorized into two types, the marginal  $R^2$  and the conditional  $R^2$ . The marginal  $R^2$  is the variance explained by fixed effects. The conditional  $R^2$  is the variance explained by both the fixed effects as the random effect Fields.

Test variable	$R^2$ by fields	$R^2$ by residuals	$R^2$ model: marginal variance	$R^2$ model: conditional variance
RGR	Int= 0.109 slope= 0.064	0.267	0.394	0.633
logSLA	0.011	0.019	0.193	0.483

LMR	Int= 0.001 Slope= 0.002	0.009	0.147	0.376
LAR	Int=23.47 Slope= 30.15	192.43	0.046	0.253
R/S ratio	Int= 0.029 Slope=0.043	0.222	0.016	0.258
Leaf thickness	Int= 0.00 Slope= 0.00	0.000	0.334	0.548

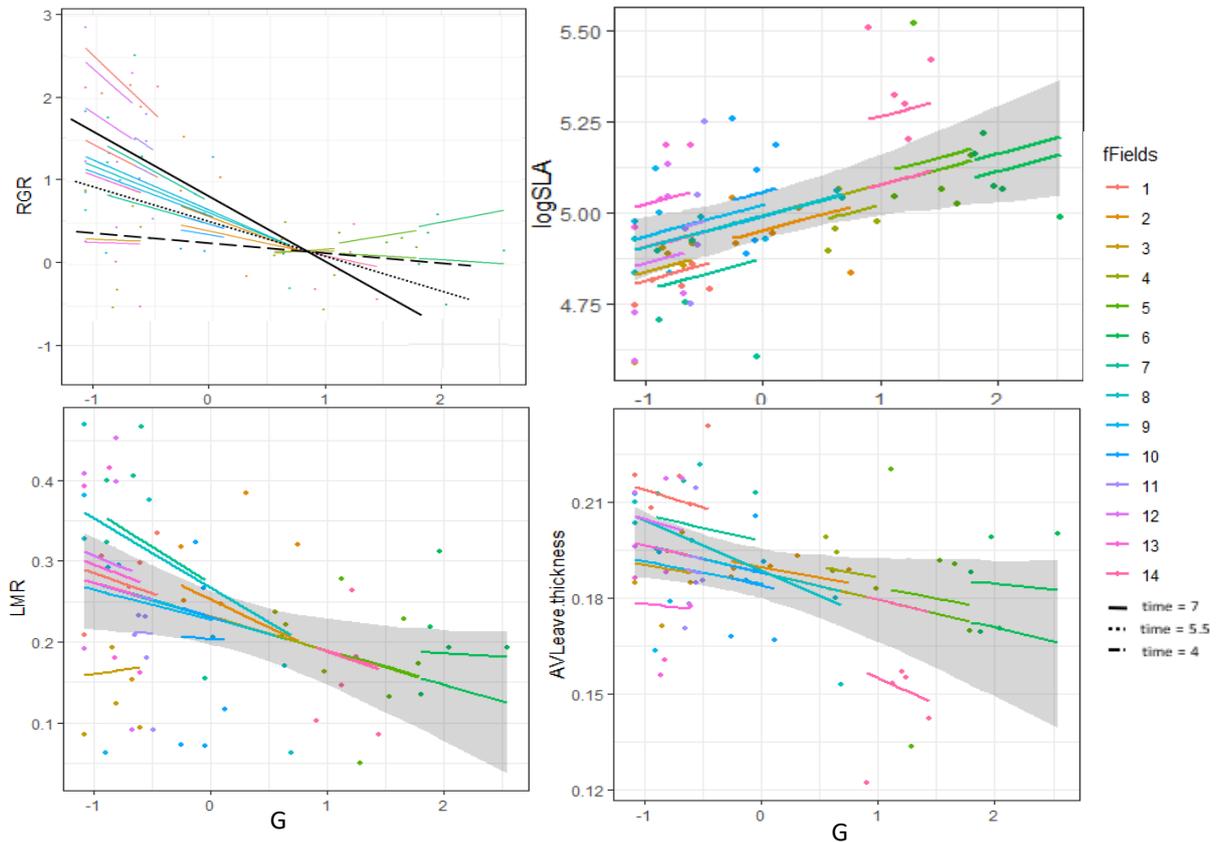


Figure 3. Prediction of the relationship between the standardized variable  $G$  and RGR,  $\log SLA$ , LMR and leaf thickness with random intercepts and random slopes for the different fields. The different colours indicate different fields. The black lines visualize the interaction effect between  $G$  and a secured amount of time: time=7 for the straight line, time=5.5 for the dotted line and time=4 for the striped line. The grey area is the 95% confidence interval of the community level prediction.

Light had a positive effect on RGR with an average increase of  $0.7 \text{ g g}^{-1} \text{ year}^{-1}$  (fig 3). Field 1 and 12 had a different intercept and field 5 and 6 a different slope than the average. Appendix 7 and 13 show low light availability in fields 5 and 6 and time since planting was only 4 months. In field 1 and 12 light availability was high. Table 2 shows quite some variation was explained by the model. It was not possible with ggplot to show the confidence interval around this model, because of the interaction effect. Light had a negative effect on SLA (fig 3). Table 2 shows quite some variation was explained by the model. Deviant field 14 show low light conditions, 7 and 13 high light conditions. Unexpectedly, light had a positive effect on LMR Field 10, 13, 14 are very similar to the average, while field 3, 6 and 7 are more different. Neither LAR or R/S ratio were significantly driven by any of the explanatory variables and the data variance explained only a small fraction of the models (table 2). Light had a positive effect on leaf thickness trough the interaction terms. Because the slopes of the interaction terms are very small, it was possible to show the confidence interval. Variation was explained for a large fraction by the model.

### Hypothesis 1 part 2: Simultaneous relationships

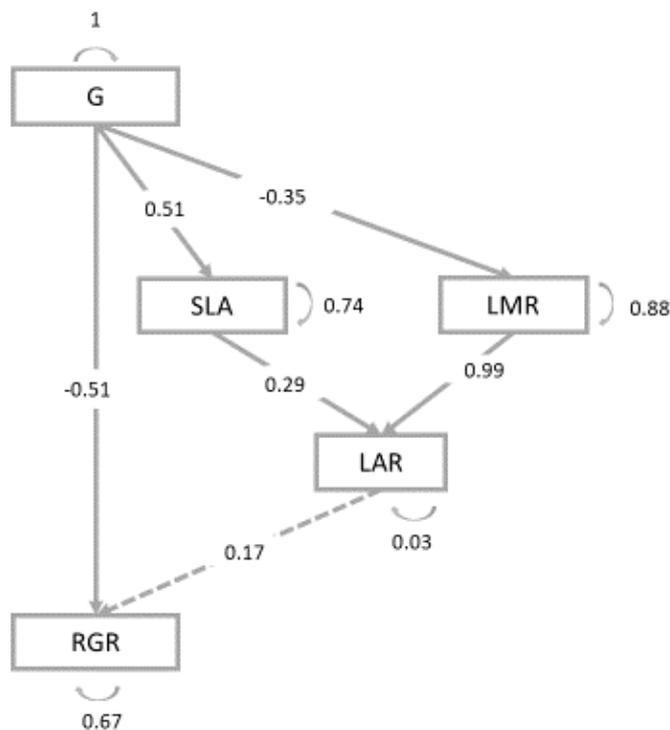


Figure 4. Result of the path analysis. The full lines indicate significant relations ( $p < 0.05$ ) and the dotted lines indicate non-significant relations. The numbers around the arrows are standardized path coefficients. This model explains 32% of the RGR ( $R^2$ ).

Standardized path coefficients with absolute values  $< 0.10$  indicating a small effect for LAR on RGR, 0.30-0.50 a medium effect for light on LMR and SLA on LAR and  $> 0.5$  a large effect for light on SLA and RGR and LMR on LAR (fig 4 and appendix 12). The estimated total effect of G on RGR is  $-0.54$  ( $-0.51 + (-0.35 * 0.99 * 0.17) + (0.51 * 0.29 * 0.17)$ ). Thus, in terms of standardized units, the direct effect of G on RGR was lower than the total effect of G on RGR ( $-0.51$  vs  $-0.54$ ).

The SEM calculated the standardized regression coefficient or  $R^2$  for those four parameters: for SLA: 0.269, LMR: 0.113, LAR: 0.967 and for RGR: 0.318. It is the explained variance, a measure of how strongly each predictor variable in the model influences the dependent variable. LAR was high because it was only influenced by two measured values. More variance was explained for RGR than SLA and LMR and more for SLA than LMR.

All the relationships were significant, except LAR ~ RGR. The Chi-square statistic robust estimator of the p-value was  $> 0.05$ : 0.067. The CFI value was  $> 0.9$ : 0.961 for the robust estimator and 0.906 for the ML estimator, indicating a good model fit.

### Hypothesis 2: Adaptation through time

Figure 5 shows that seedlings need time to adapt to environmental conditions. All the Kruskal-Wallis tests and the ANOVAs were significant, showing that there were significant differences across groups (table 3). The results of the post-hoc tests show that there were more differences between the  $t=0$  time group combinations than between the other time-group combinations. The adaptation of the biomass allocation was less strong after 4 and 5.5 months.

Table 3. Results of SPSS outcomes of the ANOVA and Kruskal-Wallis tests

Test variable	ANOVA	Kruskal-wallis	P value
Dryweight cotyl	F(3,75)=3.979		0.011
SLA		X <sup>2</sup> (3)=19.052	0.000
LMR		X <sup>2</sup> (3)=15.021	0.020
LAR		X <sup>2</sup> (3)=12.72	0.005
R/S ratio		X <sup>2</sup> (3)=16.426	0.001
Leaf thickness	F(3,75)=11.945		0.000

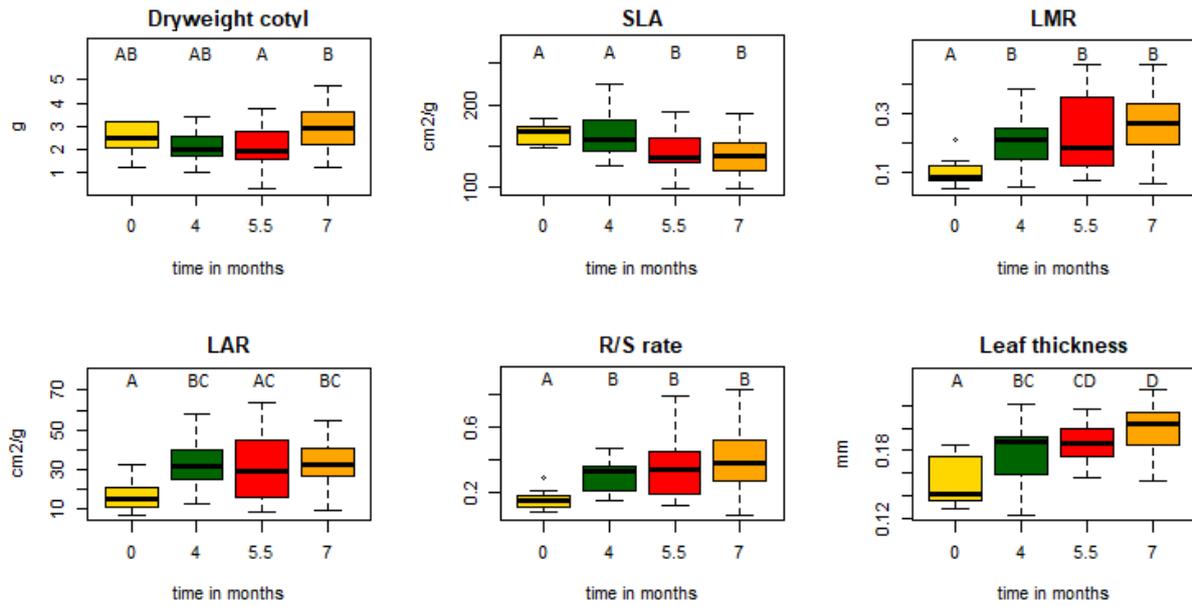


Figure 5. Boxplots of test variables (dryweight cotyledon, SLA, LMR, LAR, R/S rate and leaf thickness) and the four time groups ( $t=0, 4, 5.5, 7$ ). Different letters above the boxplots indicate the significant differences between the groups based on Post hoc tests. Similar letters mean no significant difference between median of the groups. Made in R studio version 3.5.2, letters added manually.

## Discussion

This thesis focussed on Brazil nut seedling responses after enrichment planting by comparing biomass growth and allocation under different light conditions and between seedlings growing in the nursery and in the fields. I will evaluate these responses supported by the results after evaluating the methodological strengths and limitations of this research. I will evaluate to what extent the results agree with previous studies and end with recommendations for future research and enrichment planting of *B. excelsa* seedlings.

### *Methodological strengths and limitations*

This is one of the few studies evaluating enrichment planting activities for Brazil nut trees and the first that investigated enrichment success with a growth analysis. A difficulty of research to growth analysis is to obtain permission to harvest enough seedlings and to compensate for the loss, therefore in this study sample size was smaller than recommended. Furthermore, it was not possible for most of the models to include final dry weight in the full model, because the biomass was calculated in these values. However, it would have been good to include more information on initial ontology because a certain plant form can also be the consequence of its initial plant size. Another limitation was that the ‘time since planted’ used for the RGR and time group calculations was based on collected, sometimes inexact, verbal information about planting dates from the castañeros.

For 12 seedlings a negative RGR was found, which means that biomass of the seedling was smaller than the average biomass of the seedlings from the nursery. Further inspection of these seedlings showed that all had very small or few leaves likely because of too much or too little light, or herbivores (e.g. ants or rodents) had partly eaten the seed or the roots. The weight of the cotyledon for all the seedlings with a negative RGR was smaller than the average weight of the cotyledons, which indicates that these seedlings had had a higher need for the reserves in the cotyledon. Brazil nut seedlings are unable to survive long periods in a dense understory, once their initial seed reserves have been exhausted (van Rijsoort *et al.*, 1993). Therefore, lack of light can cause slowly dying of the seedling and too much light can cause water stress and dropping of leaves. I did not investigate the effect of light conditions on the mortality of seedlings. However visual inspections showed higher seedling mortality under high light conditions.

### *Effect of irradiance on biomass growth and allocation*

As hypothesized, this study showed that light conditions were significantly positive for RGR and leaf thickness (indirect via the interaction term) and significant negative for SLA. Unexpectedly, light conditions were significantly positive for LMR and non-significant for LAR and R/S ratio.

#### *Growth*

In other studies, the RGR of *B. excelsa* seedlings also increased with higher light availability, however these values for RGR were higher than the values found in this study (Poorter, 1999; Myers *et al.*, 2000; Zuidema *et al.*, 1999; van Rijsoort *et al.*, 1993; Zuidema, 2003). In a study of Zuidema *et al.* (1999), the RGR was highest for the 100% light level. Poorter (1999) found that RGR was highest at intermediate light levels (25-50% light). In controlled experiments, *B. excelsa* seedlings growing under different light levels were found to have highest biomass in the range of 10-100% light (Poorter 1999; Zuidema, 1999). Adaptation to new environmental conditions after transplantation strongly limit the RGR.

#### *What caused the relatively low RGR compared to previous studies?*

Furthermore, after comparison with other studies, I found a positive effect of initial planting size on RGR. In other studies, initial seedling height was higher than the seedlings from this study (fig 6). The increase of growth with initial seedling size can be caused by a diminished cotyledon predation when transplanted to natural conditions, an important cause of death for small seedlings (Zuidema, 2003). On the other hand, Zuidema *et al.* (1999) found that seedlings larger than 60 cm need more time to adapt after transplantation. Zuidema *et al.* (1999) concluded that optimal seedling height when planted was 40-60 cm. Poorter (1998) and Hayashida-Oliver *et al.* (2001) found similar results (fig 6).

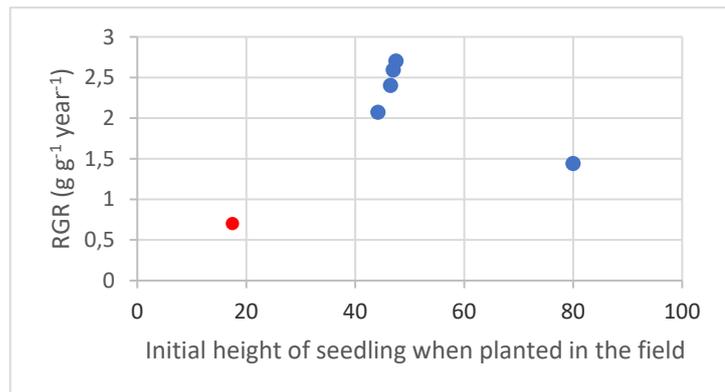


Figure 6. Relationship between RGR and initial height of seedlings when planted in the field. Points from left to right: this study (red dot), Poorter (1998), Poorter (1998), Zuidema *et al.*, (1990), Hayashida-Oliver *et al.*, (2001) and Zuidema *et al.*, (1990) (blue dots).

The MEM showed that the interaction effect of light and time also significantly influenced RGR. Because seedlings need to acclimatize in the field, initially more energy of the plant goes into changing the biomass allocation. After adaptation, more energy can be invested in the growth of the seedling. Next to light and time, for example water and nutrients can influence RGR. Zuidema & Boot (2002) showed that survival of seedlings was lower in a dry year than in a normal year. In this study, seedlings were planted between March and June. The wet season in the Madre de Dios region ends in March-April, therefore

the seedlings should all be planted April latest. I cannot exclude that there have been differences in water availability between the fields that potentially have influenced the results. Soil variables like soil moisture, soil pH and nutrients or management actions from the castañeros like fertilization, anti-herbivore measurements, fire damage, seedling origin, etcetera (Grubb, 1996) can also have influenced the results.

#### *Allocation*

The predicted average value of SLA, based on the formula of the MEM ( $146.9 \text{ cm}^2 \text{ g}^{-1}$ ) is comparable with results of other studies of tropical species, ranging around  $120 \text{ cm}^2 \text{ g}^{-1}$ . For temperate species the SLA values are a lot higher, indicating a higher tolerance and adaptation to lower light conditions (Tyree *et al.*, 1999). These results are consistent with the strong correlation between SLA and leaf thickness found in this research (Pearson correlation coefficient  $r=-0.7$ ,  $p=0.000$ ). Several other studies of seedlings of other tropical tree species have shown that the SLA of leaves decrease in plants grown under full sunlight (Zuidema *et al.*, 1999; Azevedo, 2014; Popma & Bongers, 1988, Veenendaal *et al.*, 1996). In high light conditions, leaf loss was higher while the rate of leaf gain was lower or negative (Azevedo, 2014).

Unexpectedly, a significant positive effect of light was found for LMR. Gyimah & Nakao (2007) found a negative relationship between light and LMR in their study about growth responses to light of seedlings of tree tropical species. Interestingly, they found a positive relationship between light and allocation to the roots. The LMR found for high light conditions was  $0.36 \text{ g g}^{-1}$  and for low light conditions  $0.49 \text{ g g}^{-1}$ . In this study, the predicted value of LMR was on average  $0.23 \text{ g g}^{-1}$ . The positive relationship found in my study can partly be explained by the allocation of biomass to roots, because in most studies the LMR and allocation to the roots are consistent (Gyimah & Nakao, 2007). I did not find a significant effect of light on the R/S ratio. It could be that water stress or predation of ants or agoutis decreased the amount of biomass found in the roots or that I have not completely succeed to remove all the root parts when harvesting a seedling. Furthermore, the positive relationship can be explained by the leaf thickness, which increased with light availability and increases the biomass of the leaves. Kelly *et al.* (2009) also found a positive relationship between light and LMR. They investigated the growth and physiological responses of six rainforest tree species to light conditions and found the highest LMR for most of the 6 investigated rainforest tree species for high light availability (Kelly *et al.*, 2009). This study found an LMR ranging on average from  $0.34 \text{ g g}^{-1}$  for high light conditions to  $0.26 \text{ g g}^{-1}$  for low light conditions (Kelly *et al.*, 2009). In addition to these LMR values, this study also found no effect of light on biomass allocation to the roots. This might explain the similarity of LMR values between my study and the study of Kelly *et al.* (2009).

The trend of LAR and light conditions found in my study is not similar with results of other studies. In a study of Popma & Bongers (1988), tropical rain forest species showed a negative effect of light on LAR. Popma & Bongers (1988) however found differences between species in LAR, depending on the shade tolerance of the species. The relationship between light and LAR can be explained by the behaviour of its two components: SLA and LMR, both showing a different trend with light. Values of LAR based on this study ranged between  $39.35 \text{ cm}^2 \text{ g}^{-1}$  for high light conditions and  $26.83 \text{ cm}^2 \text{ g}^{-1}$  for low light conditions. For Poorter (1999), values for high light levels were similar, however the LAR value for low light conditions were very different:  $31 \text{ cm}^2 \text{ g}^{-1}$  and  $64.5 \text{ cm}^2 \text{ g}^{-1}$  respectively.

Popma and Bongers (1988) found similar results for R/S ratio as this study. In their study to the growth and biomass allocation of Hawaiian rainforest species of Pattison *et al.* (1998), R/S ratio did not show consistent trends across light treatment for any of the species except one. Values ranged between 0.1 and 0.45. My values of R/S ratio were on average 0.36. Low values of R/S ratio can indicate conditions of high nutrient availability or a lack of competition.

#### ***Simultaneous relations***

Multiple regression like MEMs are ideal if variables are all directly measured. Clear hypotheses with enough evidence to support the interpretation that a variable has a causal effect on another variable are necessary. In this study, this prerequisite can be met. However, not all the relationships analysed with MEM were directly measured as shown by the conceptual diagram. Furthermore, latent variables (unmeasured, underlying causes) can influence the relationships between values (Grace, 2006). Therefore, SEM can provide insights into a whole system of interactions with direct and indirect interactions and relative strengths of pathways. SEM represents a different way of analysing data by examining networks of causal relationships. Therefore, SEMs and MEMs are difficult to compare (Grace, 2006). In this study, the direction of the relationships was similar for both models, even as most of the significant relationships. SEM found additionally a non-significant relationship between LAR and RGR and the indirect relationship between G and LAR was significant. Some coefficients of the MEM correspond to path coefficients and others do not. An explanation for the differences can be the influence of the latent variables. It is not realistic to hold  $\text{SLA} \sim \text{LAR}$  constant when evaluating  $\text{LMR} \sim \text{LAR}$  because LAR it was directly calculated from SLA. Therefore, the collective effects are of interest. Another explanation is that SEM only modelled G while MEM also included time as explanatory variable. In the SEM, G explains 32% of the RGR, which means that other explaining variables explain the other 68% of RGR. The match between model and data indicated by the chi-square value of the SEM in this study indicates that the conceptual diagram was correct.

#### ***Adaptation after transplantation***

The differences between the seedlings in the nursery and the seedlings in the field can be explained by a lack of light and an abundance of water in the nursery. In addition to this, the nursery from the IIAP plants the *B. excelsa* seedlings in v-shaped

tubes. In these tubes, limited space is available to develop a suitable root system. It might be better to give the seedling more soil space to be able to develop a sufficient root system. The boxplot of the cotyledon shows that the dry weight of the cotyledon first decreases and then increases in the last time group. Zuidema *et al.* (1999) showed in their experiment that low values of RGR in the shaded treatments were accompanied by positive values of relative use of the cotyledon, indicating a net use of the cotyledon reserve. This indicates that in my research the cotyledon reserves were used for biomass allocation. After some time, lignification of the cotyledon causes the dry weight to increase again.

Similar to my results, Villar-Salvador *et al.* (2004) did an experiment with drought hardening of seedlings and concluded that outplanting performance was related to changes in plant size and biomass allocation induced by nursery treatments. A high R/S ratio and small shoot sizes have been considered drought resistance traits. Other studies also found that the morphology and especially the root system of seedlings was sensitive to irradiation and watering in the nursery growing period (Andrés *et al.*, 2011; Davis & Jacobs, 2005). Grossnickle (2012) argues that it is important that nurseries create seedlings with plant attributes that allow for the best chance of success once a seedling is planted in the field. He also found that even though seedling losses are in many situations attributed to animal grazing, disease or insects, in many cases the real cause is planting poor quality seedlings.

### ***Enrichment planting***

Over the long term, enrichment planting of a NTFP may serve forest conservation while supporting human economic activities and gives farmers an alternative to land conversion (Belcher & Schreckenberg, 2007). Examples of successful NTFP planting are rattan in combination with rubber trees as support in Malaysia and Indonesia or Açaí in the Amazon, which has greatly increased the value of forests there (Gunter *et al.*, 2011; Paquette *et al.*, 2009). However, enrichment planting also includes high costs, therefore in some situations the benefits may be unclear.

### ***Recommendations for future research***

In this research, I only investigated enrichment planting performance for light conditions and time in the field and discussed that also initial height of seedlings when planted, date of planting and other explaining variables as water availability, soil and silvicultural interventions by the castañeros are important (Kainer *et al.*, 1998). Therefore, in future research it is important to also include the effect of those other explaining variables on the performance of *B. excelsa*. Also, optimal height growth and mortality are important to include to investigate performance of seedlings. Finally, research is needed to the best preparing conditions in nurseries for future planting.

### ***Management advises***

It is important to understand the requirements of *B. excelsa* seedlings to light conditions because the responses of the plants to those conditions determine the success of enrichment planting. *B. excelsa* seedlings perform better under high light conditions, however the optimum might be under the maximal light conditions. In this study, seedlings in fields 1 and 12 showed the highest RGR, both with high light conditions of  $G < 2$  (appendix 13). This were the best fields for enrichment planting. Literature states that the optimal height of *B. excelsa* seedlings when planted in the fields was 40-60 cm. I advise to wait with transplanting until this optimal height is reached. Furthermore, the seedlings need to be prepared to transplantation to minimize adaptation in biomass allocation and mortality rates. Subjecting the seedlings to mild water stress can improve the acclimatisation responses. It might be better not to plant the seedlings in the v-shaped tubes with limited space for root development. Last, management strategies like planting date, planting practices, site preparation and vegetation management are important for the success of enrichment planting and moreover good monitoring will improve management practices.

## **Conclusions**

The results of this thesis have yielded two important results. First, light increases the biomass growth and allocation of *B. excelsa* seedlings. Therefore, optimal light conditions are crucial for enrichment planting success. Second, seedlings from the nursery are not well prepared to the environmental conditions in the field. For optimal success, seedlings should have an optimal length and biomass allocation before transplantation. In this way, enrichment planting of *B. excelsa* can increase the value of the forest while simultaneously supporting social development of people dependent on the Brazil-nut market and thus contributing to the conservation of natural ecosystems.

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## References

- Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics international*, 11(7), 36-42.
- Andrés, P., Salgado, C., & Espelta, J. M. (2011). Optimizing nursery and plantation methods to grow *Cedrela odorata* seedlings in tropical dry agroecosystems. *Agroforestry systems*, 83(2), 225-234.
- Azevedo, G. F. D. C. (2014). Photosynthetic parameters and growth in seedlings of *Bertholletia excelsa* and *Carapa guianensis* in response to pre-acclimation to full sunlight and mild water stress. *Acta Amazonica*, 44(1), 67-77.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.
- Belcher, B., & Schreckenberg, K. (2007). Commercialisation of non-timber forest products: A reality check. *Development Policy Review*, 25(3), 355-377.
- Belcher, B. M., & Vantomme, P. (2003). What isn't an NTFP?. *The International Forestry Review*, 5(2), 161-168.
- Canham, C. D., Berkowitz, A. R., Kelly, V. R., Lovett, G. M., Ollinger, S. V., & Schnurr, J. (1996). Biomass allocation and multiple resource limitation in tree seedlings. *Canadian Journal of Forest Research*, 26(9), 1521-1530.
- Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., ... & Kinzig, A. P. (2012). Biodiversity loss and its impact on humanity. *Nature*, 486(7401), 59-67.
- Chambers, J. M., Freeny, A and Heiberger, R. M. (1992) Analysis of variance; designed experiments. Chapter 5 of *Statistical Models in S* eds J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole
- Chazdon, R. L. (2014). *Second growth: the promise of tropical forest regeneration in an age of deforestation*. University of Chicago Press.
- Chiriboga Arroyo, F. (2018, April 20). E-mail.
- Chunhieng, T., Pétritis, K., Elfakir, C., Brochier, J., Goli, T., & Montet, D. (2004). Study of selenium distribution in the protein fractions of the Brazil nut, *Bertholletia excelsa*. *Journal of agricultural and food chemistry*, 52(13), 4318-4322.
- Climate-Data (2015). Climate of Madre de Dios. Retrieved from <https://en.climate-data.org/region/1237/>
- Davis, A. S., & Jacobs, D. F. (2005). Quantifying root system quality of nursery seedlings and relationship to outplanting performance. *New Forests*, 30(2-3), 295-311.
- Dion, P. A. (2008). Interpreting structural equation modeling results: a reply to Martin and Cullen. *Journal of Business Ethics*, 83(3), 365-368.
- Eager, Christopher D. (2017). standardize: Tools for Standardizing Variables for Regression in R. R package version 0.2.1. <https://CRAN.R-project.org/package=standardize>
- Epskamp, S. (2015). semPlot: Unified visualizations of Structural Equation Models. *Structural Equation Modeling. Structural Equation Modeling*, 22(3), 474-483.
- Fisher, B., Turner, R. K., & Morling, P. (2009). Defining and classifying ecosystem services for decision making. *Ecological economics*, 68(3), 643-653.
- Food and Agriculture Organization. (2018). FAOSTAT. Retrieved from <http://www.fao.org/faostat/en/#data/TP>.
- Galia Selaya, N., Zuidema, P. A., Baraloto, C., Vos, V. A., Brienen, R. J., Pitman, N., ... & Vasquez Colomo, G. H. (2017). Economically important species dominate aboveground carbon storage in forests of southwestern Amazonia. *Ecology and Society*, 22(2).
- Grace, J. B. (2006). *Structural equation modeling and natural systems*. Cambridge University Press.
- Grossnickle, S. C. (2012). Why seedlings survive: influence of plant attributes. *New Forests*, 43(5-6), 711-738.
- Grubb, P. J., Lee, W. G., Kollmann, J., & Wilson, J. B. (1996). Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*. *Journal of Ecology*, 827-840.
- Guariguata, M. R., Cronkleton, P., Duchelle, A. E., & Zuidema, P. A. (2017). Revisiting the 'cornerstone of Amazonian conservation': a socioecological assessment of Brazil nut exploitation. *Biodiversity and Conservation*, 26(9), 2007-2027.

- Gunter, S., Weber, M., Stimm, B., & Mosandl, R. (2011). *Silviculture in the Tropics*. Springer.
- Gyimah, R., & Nakao, T. (2007). Early growth and photosynthetic responses to light in seedlings of three tropical species differing in successional strategies. *New Forests*, 33(3), 217.
- Hayashida-Oliver, Y., Boot, R. G., & Poorter, L. (2001). Influencia de la disponibilidad de agua y luz en el crecimiento y la morfología de plantines de *Swietenia macrophylla*, *Cedrela odorata* y *Bertholletia excelsa*. *Ecología en Bolivia*, 35, 51-60.
- Holl, K. D., & Aide, T. M. (2011). When and where to actively restore ecosystems?. *Forest Ecology and Management*, 261(10), 1558-1563.
- Kainer, K. A., Duryea, M. L., Costa de Macêdo, N., & Williams, K. (1998). Brazil nut seedling establishment and autecology in extractive reserves of Acre, Brazil. *Ecological Applications*, 8(2), 397-410.
- Kainer, K. A., Wadt, L. H., & Staudhammer, C. L. (2007). Explaining variation in Brazil nut fruit production. *Forest Ecology and Management*, 250(3), 244-255.
- Kelly, J., Jose, S., Nichols, J. D., & Bristow, M. (2009). Growth and physiological response of six Australian rainforest tree species to a light gradient. *Forest Ecology and Management*, 257(1), 287-293.
- Lambers, H., Chapin, F. S., & Pons, T. L. (2008). Photosynthesis. In *Plant physiological ecology* (pp. 11-99). Springer, New York, NY.
- Lieberman, M., Lieberman, D., & Peralta, R. (1989). Forests are not just Swiss cheese: canopy stereogeometry of non-gaps in tropical forests. *Ecology*, 70(3), 550-552.
- Moraes, V. D. F., Müller, C. H., De Souza, A. G. C., & Antonio, I. C. (1994). Native fruit species of economic potential from the Brazilian Amazon. *Embrapa Amazônia Ocidental-Artigo em periódico indexado (ALICE)*.
- Myers, G. P., Newton, A. C., & Melgarejo, O. (2000). The influence of canopy gap size on natural regeneration of Brazil nut (*Bertholletia excelsa*) in Bolivia. *Forest Ecology and Management*, 127(1-3), 119-128.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4(2), 133-142.
- Oberski, D. (2014). lavaan. survey: An R package for complex survey analysis of structural equation models. *Journal of Statistical Software*, 57(1), 1-27.
- Pattison, R. R., Goldstein, G., & Ares, A. (1998). Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. *Oecologia*, 117(4), 449-459.
- Pandey, R., Paul, V., Das, M., Meena, M., & Meena, R. C. (2017). Plant Growth Analysis. Manual of ICAR Sponsored Training Programme for Technical Staff of ICAR Institutes on “Physiological Techniques to Analyze the Impact of Climate Change on Crop Plants”, 103.
- Paquette, A., Hawryshyn, J., Senikas, A. V., & Potvin, C. J. (2009). Enrichment planting in secondary forests: a promising clean development mechanism to increase terrestrial carbon sinks. *Ecology and Society*.
- Peña-Claros, M., Boot, R. G., Dorado-Lora, J., & Zonta, A. (2002). Enrichment planting of *Bertholletia excelsa* in secondary forest in the Bolivian Amazon: effect of cutting line width on survival, growth and crown traits. *Forest Ecology and Management*, 161(1-3), 159-168.
- Peres, C. A., Baider, C., Zuidema, P. A., Wadt, L. H., Kainer, K. A., Gomes-Silva, D. A., ... & Gribel, R. (2003). Demographic threats to the sustainability of Brazil nut exploitation. *Science*, 302(5653), 2112-2114.
- Pierce, A., Shanley, P., & Laird, S. (2003). Certification of non-timber forest products: limitations and implications of a market-based conservation tool. In *international conference on rural livelihoods, forests and biodiversity*, Bonn, Germany (pp. 19-23).
- Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: a quantitative review. *Functional Plant Biology*, 27(12), 1191-1191
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), 30-50.
- Poorter, L. (1998). Seedling growth of Bolivian rain forest tree species in relation to light and water availability.
- Poorter, L. (1999). Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Functional ecology*, 13(3), 396-410.

- Popma, J., & Bongers, F. (1988). The effect of canopy gaps on growth and morphology of seedlings of rain forest species. *Oecologia*, 75(4), 625-632.
- Savalei, V. (2014). Understanding robust corrections in structural equation modelling. *Structural Equation Modeling: A Multidisciplinary Journal*, 21(1), 149-160.
- Schermelleh-Engel, K., Moosbrugger, H., & Müller, H. (2003). Evaluating the fit of structural equation models: Tests of significance and descriptive goodness-of-fit measures. *Methods of psychological research online*, 8(2), 23-74.
- SUSTAIN (n.d.). From the Peruvian Amazon to consumers in Switzerland. BRAZIL NUTS. Retrieved April 04, from <https://www.sustain-forest.org/>
- Tambopata National Reserve (2019). Retrieved from <https://www.perunature.com/about-tambopata/tambopata-national-reserve/>
- Tyree, M. T., Sobrado, M. A., Stratton, L. J., & Becker, P. (1999). Diversity of hydraulic conductance in leaves of temperate and tropical species: possible causes and consequences. *Journal of Tropical Forest Science*, 47-60.
- Tyree, M. T., Velez, V., & Dalling, J. W. (1998). Growth dynamics of root and shoot hydraulic conductance in seedlings of five neotropical tree species: scaling to show possible adaptation to differing light regimes. *Oecologia*, 114(3), 293-298.
- van Rijsoort, J., Ugueto, S., & Zuidema, P. (1993). The Brazil nut tree (*Bertholletia excelsa*): population structures in tropical rain forest and growth response of seedlings to different light intensities (Doctoral dissertation, M. Sc. thesis, Utrecht University, Utrecht).
- Veenendaal, E. M., Swaine, M. D., Lecha, R. T., Walsh, M. F., Abebrese, I. K., & Owusu-Afriyie, K. (1996). Responses of West African forest tree seedlings to irradiance and soil fertility. *Functional Ecology*, 10(4), 501-511.
- Vernon, A. J., & Allison, J. C. S. (1963). A method of calculating net assimilation rate. *Nature*, 200(4908), 814.
- Villar-Salvador, P., Planelles, R., Oliet, J., Peñuelas-Rubira, J. L., Jacobs, D. F., & González, M. (2004). Drought tolerance and transplanting performance of holm oak (*Quercus ilex*) seedlings after drought hardening in the nursery. *Tree physiology*, 24(10), 1147-1155.
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York
- Wildscreen Arkive (n.d.) Brazil-nut tree. Retrieved April 04, 2018, from <https://www.arkive.org/brazil-nut-tree/bertholletia-excelsa/>
- Zuidema, P. A., & Boot, R. G. (2002). Demography of the Brazil nut tree (*Bertholletia excelsa*) in the Bolivian Amazon: impact of seed extraction on recruitment and population dynamics. *Journal of Tropical Ecology*, 18(1), 1-31.
- Zuidema, P. A., Dijkman, W., & Rijsoort, J. V. (1999). Crecimiento de plantines de *Bertholletia excelsa* HBK em función de su tamaño y la disponibilidad de luz. *Ecologia em Bolívia*, 33, 23-35.
- Zuidema, P. A. (2003). Ecology and management of the Brazil nut tree (*Bertholletia excelsa*). *Promab*.

## Appendix 1

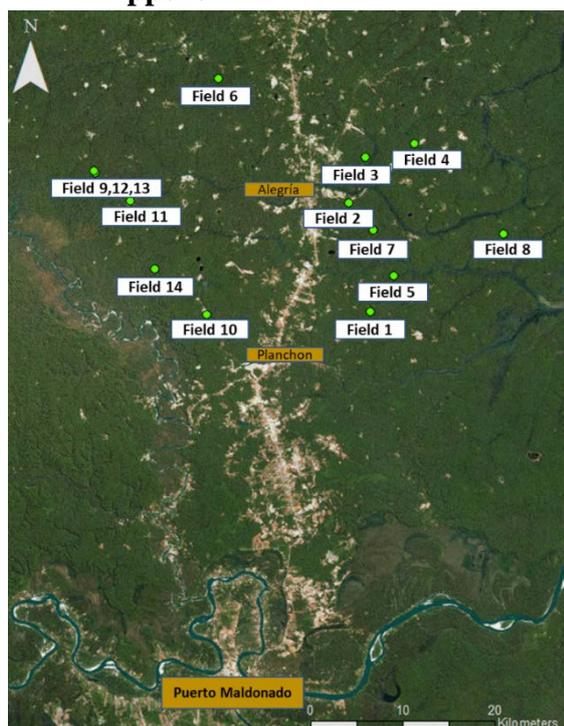


Figure i. Map with the locations of the measured fields in the Madre de Dios region of Peru. Created with ArcMap (2019).

## Appendix 2

Table i. Information about the 14 different fields: time the seedlings grew in the fields on the moment of harvest, the location and the owner.

Field	Time in the field (months)	GPS-location	NAME CASTANERO
1	7	12°14'04.33"S 69°03'36.22"W	Manuel Gamarra Fuentes
2	5	12°07'49.1"S 69°04'53.65"W	Justa Castro Quispe
3	6	12.085977°S, 069.065279°W	Sara Hurtado Orosco
4	3	12°04'23.8"S 69°01'03.27"W	Manuel Escalante Huaman y Gulio Instalaron
5	4	12°12'00.65"S 69°02'15.81"W	René vargas pineda
6	4	12°00'38.83"S 69°12'30.66"W	Basilides mamani puma
7	5.5	12°09'23.11"S 69°03'28.06"W	Eulogio quispe Chani
8	6.5	12°09'35.76"S 68°55'49.23"W	Luisa Ohoa Puma
9	6.5	12°06'02.51"S 69°19'45.28"W	Domingo quispe pinedo
10	5.5	12°14'14.2"S 69°13'13.7"W	carlos moreno fuller
11	7	12°07'40.6"S 69°17'41.7"W	Miliska guerra vargas
12	8	12°05'58.7"S 69°19'50.7"W	Franklin quispe gomes
13	6	12°05'53.9"S 69°19'48.0"W	Paulino quispe Ramirez
14	5	12°11'36.4"S 69°16'14.1"W	Felicitas Macochoa

### **Appendix 3**

#### **ACTA DE COMPROMISO**

El **PROYECTO SUSTAIN**, aliado del Proyecto Castañas de Profonanpe, se compromete con el socio castañoero que suscribe esta ACTA DE COMPROMISO, a:

- Entregarle 3 plántones por cada plánton retirado de su parcela agroforestal (dando un total de 15 plántones), durante los meses de diciembre 2018 y febrero 2019.
- Sembrar los 15 plántones entregados en los sitios que indique el socio, ubicados dentro de la misma parcela agroforestal.

En señal de conformidad, firman esta acta de compromiso, el día \_\_\_\_ de \_\_\_\_ de \_\_\_\_:



Fidel Chiriboga  
Proyecto Sustain

Socio Castañoero:

#### **ACTA DE COMPROMISO**

El **PROYECTO SUSTAIN**, aliado del Proyecto Castañas de Profonanpe, se compromete con el socio castañoero que suscribe esta ACTA DE COMPROMISO, a:

- Entregarle 3 plántones por cada plánton retirado de su parcela agroforestal (dando un total de 15 plántones), durante los meses de diciembre 2018 y febrero 2019.
- Sembrar los 15 plántones entregados en los sitios que indique el socio, ubicados dentro de la misma parcela agroforestal.

En señal de conformidad, firman esta acta de compromiso, el día \_\_\_\_ de \_\_\_\_ de \_\_\_\_:



Fidel Chiriboga  
Proyecto Sustain

Socio Castañoero:

## Appendix 4



Nombre: Jiska Schaaf  
Edad: 24  
Estudio: Msc Conservación del Bosque  
Universidad, país: Universidad de Wageningen, Holanda

Propósito de la investigación: Para mi tesis de maestría investigo las condiciones ideales de iluminación para las plántulas de nuez de Brasil. Para eso mido las condiciones de luz y hago un análisis de crecimiento. Para eso necesito algunas plantas. Utilizo estas plántulas para calcular cuánto han crecido las plántulas desde la siembra y bajo qué condiciones de iluminación tienen que ajustarse más. La cantidad de plántulas que invierten en el crecimiento de la hoja o en la raíz depende de las condiciones de iluminación. También mediré el grosor de la hoja de varias plántulas. Los resultados de mi investigación contribuirán a un mejor conocimiento sobre las condiciones ideales para plantar plántulas de árboles de castaña.

Esta investigación es en colaboración con: SUSTAIN, Fondo de Promoción de las Áreas Naturales Protegidas del Perú (PROFONANE), Instituto de Investigaciones de la Amazonía Peruana (IIAP) y la Universidad de Wageningen.



## Appendix 5

### Random number table

85679 69448 94726 99484 34381 76609 89892 62932 97231 35243 64286 43198 57796
95424 70862 83857 07648 97251 84861 76448 07786 75128 25935 99279 71164 12830
71259 41524 17188 85447 62969 30051 92650 13036 42600 36149 09773 64515 49609
43870 63584 28030 68080 30586 76016 22254 90447 72749 82084 38624 57684 30179
74342 63203 37959 66455 52387 64322 61259 74194 68371 16725 54067 31623 31539
25012 08613 85745 44707 91712 23484 35741 65336 84274 36910 64216 12530 08647
95842 39908 25139 23178 92446 64778 51152 36401 29819 06625 80886 55138 14293
90065 79287 60042 13592 09790 25094 84591 97869 34692 38848 66265 02711 89126
91342 39914 96046 57523 49344 48586 16144 89038 71832 95295 01538 84481 39123
95617 15636 68719 09237 23111 90140 74213 87343 26515 90926 04963 72693 39675
58931 10095 38932 92757 74358 65906 45542 41853 59466 69386 85033 56680 75983
11582 30773 67334 95982 23991 29367 58910 77700 02374 25453 70462 04180 66299
78907 81308 53707 85926 19367 33415 42326 23898 89989 84575 96886 76681 90193
71174 20698 98844 59140 17886 55158 76730 94389 63531 69650 47521 05169 71552
77844 52005 97193 48553 13482 16084 03816 79018 72062 85039 49171 22566 82437
76444 44575 24620 54039 99126 11117 26843 00931 59283 00205 98138 78154 82656
11904 20784 36914 12319 91959 15543 93059 71282 47507 86830 07143 72111 43359
07540 25772 59538 77036 68966 20562 97462 16216 75432 70516 94709 61452 74008
16095 54216 58261 13709 34349 47117 60068 58358 23549 36787 45395 93700 84302
66303 23382 95017 42661 59920 82683 69711 79826 87979 53332 78363 36700 49116
69723 65672 04778 98185 18645 30815 07038 46167 51569 89917 10065 66205 10494
85820 70282 02669 27210 09950 84805 37176 03333 71245 07465 90039 05923 01754
13481 66356 10345 14077 14791 09269 03715 06405 95741 29057 90321 74553 56969
37936 63673 82739 34693 50414 77537 93225 19840 94283 21965 53859 32493 61521
05567 45805 86961 25911 40927 99063 91649 44789 06662 86941 61713 37920 06662
83210 22127 90204 71074 05162 32327 90052 48725 67819 92212 71678 37725 61044
23950 66788 25847 44072 57016 20764 07939 93746 83048 10142 79412 70658 62713
15818 48749 88031 63272 13527 63117 21097 79157 54589 03190 82904 45981 95554
71409 50079 75891 29286 01372 26805 47714 05130 36740 98374 68602 35555 82864
42048 69272 14991 06260 42926 43991 43408 79238 07953 21461 59192 18968 07280
67387 34428 40716 02746 51165 82634 65412 13603 09253 92291 22345 79306 52430
22714 69516 78060 67305 62601 98776 32962 76807 13585 68860 93116 14735 19714
22475 60684 59073 84138 39114 65339 79365 70805 60962 41834 10753 43173 13778
39249 81146 60541 04643 36572 04008 34518 47646 13710 05902 14541 26541 01329
41508 87572 09567 79409 27763 56882 05496 36614 22337 20199 20815 37124 64786
34499 94627 24077 96478 49655 70849 57136 04650 88288 56975 07964 68997 76327
06355 08601 32397 97442 52351 75183 62532 48842 66778 57096 44644 76889 83378
98985 65296 65474 60622 48832 15072 01825 50737 41430 76974 66332 53648 66791
71993 69266 47597 93509 67416 95571 25037 77525 03791 05149 49827 96225 07125
25042 90421 44814 70259 81750 60881 36315 72886 80338 35926 81673 42976 20346
78437 86990 12420 77724 86784 53116 84979 26929 78806 49218 20311 96077 34609
08884 35337 34733 50500 38824 02301 91160 77517 31268 35740 77496 87332 04195
68161 65939 93272 60943 74744 07427 03415 20515 04538 59559 69378 53207 63948
01311 02400 61345 14510 56869 82878 25072 26920 77313 24053 75267 29679 94210
69489 85506 08922 42113 41965 66082 05153 70182 62577 77351 89480 05694 38936

## Appendix 6

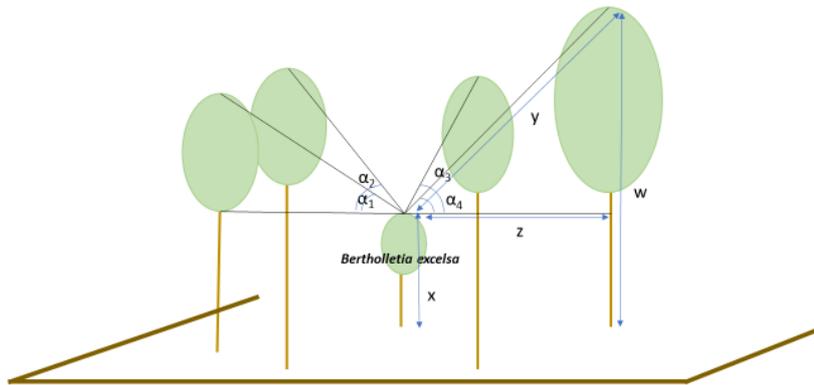


Figure ii. Method used to calculate the light intensity that the *B. excelsa* seedling receives.

## Appendix 7

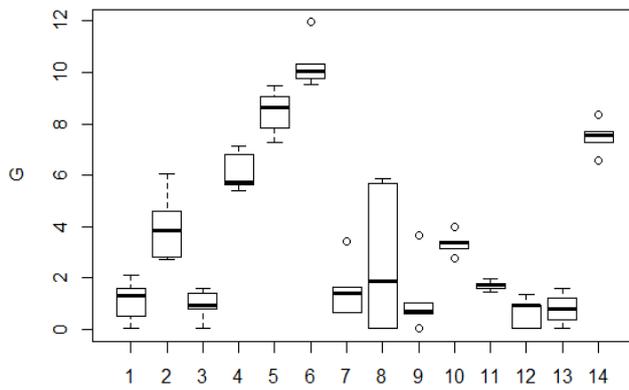


Figure iii. Boxplot of the light conditions ( $G$ ) for the 14 investigated fields in the Madre de Dios region, Peru. The outcome of the post-hoc test is shown in appendix 4.

## Appendix 8

Table ii. Outcome of the post-hoc test for the different light conditions in the field. The X indicate significant differences.

	Field 1	Field 2	Field 3	Field 4	Field 5	Field 6	Field 7	Field 8	Field 9	Field 10	Field 11	Field 12	Field 13
Field 2	X												
Field 3		X											
Field 4	X	X	X										
Field 5	X	X	X	X									
Field 6	X	X	X	X	X								
Field 7		X		X	X	X							
Field 8	X		X	X	X	X							
Field 9		X		X	X	X		X					
Field 10	X		X	X	X	X	X		X				
Field 11		X		X	X	X				X			
Field 12		X		X	X	X				X			
Field 13		X		X	X	X				X			
Field 14	x	x	x			x	x	X	x	x	x	x	X

## Appendix 9

Table iii. Outcome of the Pearson correlation test between the explanatory variables

Explanatory variables	Pearson correlation coefficient
Time ~ G	-0.76
Biomass ~ G	-0.51
Biomass ~ time	0.55

## Appendix 10

Table iv. Starting models for the MEMs

Test variable	Starting model
RGR	lmer(RGR~SG*Stime +(SG fFields)
Log SLA	lmer(logSLA~SG*Stime +(SG fFields)
LMR	lmer(LMR~SG*Stime +(SG fFields)
LAR	lmer(LAR~SG*Stime +(SG fFields)
Log R/S ratio	lmer(logRrate~SG*Stime +(SG fFields)
Leaf thickness	lmer(AVLeave.thickness~(SG*SCALDWTOT)+(Stime*SCALDWTOT)+(SG*Stime) +(SG fFields)

## Appendix 11

Table v. Outcomes of the Shapiro-Wilk normality tests for the 6 models. Models are normally distributed when the outcome is not significant.

Y	Shapiro-Wilk normality test
RGR	0.9844
logSLA	0.4491
LMR	0.2885
LAR	0.7742
Log R/S ratio	0.2703
Leaf thickness	0.8432

## Appendix 12

Table vi. Outcome of the SEM

Relation	Est. std.	Std. err.	z-value	Pr> t	ci.lower	ci.upper
Light→RGR	-0.512	0.114	-4.478	0.000	-0.736	-0.288
Light→SLA	0.511	0.109	4.689	0.000	0.297	0.724
Light→LMR	-0.349	0.130	-2.681	0.007	-0.604	-0.094
SLA→LAR	0.289	0.045	6.384	0.000	0.200	0.378
LMR→LAR	0.993	0.025	40.49	0.000	0.945	1.041
LAR→RGR	0.174	0.124	1.407	0.160	-0.068	0.417
SLA~SLA	0.739	0.111	6.643	0.000	0.521	0.957
LMR~LMR	0.878	0.091	9.660	0.000	0.700	1.056
LAR~LAR	0.033	0.009	3.525	0.000	0.015	0.051
RGR~RGR	0.672	0.111	6.029	0.000	0.453	0.890
G~G	1	0	-	-	1	1

## Appendix 13



*Figure iv. Field 1, 6 and 12 respectively*