Introduction of native tree species into degraded Cerrado vegetation

Introdução de espécies arbóreas nativas em vegetação degradada de Cerrado

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Abstract: Experimental restoration using tree seedlings is a common strategy for accelerating succession on degraded post-agricultural land formerly occupied by Cerrado vegetation. Seedling growth in degraded tropical lands is constrained by various factors. The goal of this study was to evaluate the seedling growth and survival of seven native tree species used to accelerate forest recovery in a gully area with stressful environmental conditions. The experimental design involved fenced and unfenced blocks, presence and absence of fertilization and use of an adhesive to prevent ant herbivory (four treatments with four replicates). Seedlings were planted in December 2006 and collection of data on seedling basal diameter, height, mortality and herbivory started on January 24th 2007 and continued every three months, until final data collection on January 31st 2009 (9 measurement dates). Overall seedling survival was 38 % and protecting seedlings did not influence growth, but seedlings grew faster in response to the addition of fertilizer containing N, P and K. The use of the adhesive Tanglefoot to exclude leaf cutter ants had no influence on growth. Fencing reduced seedling mortality, but combining fencing with Tanglefoot did not. Nutrient availability limits seedling growth and survival in the gully. Direct planting of seedlings of native trees may accelerate succession in degraded Cerrado lands subject to interventions that overcome constraints on seedling growth and survival.

Keywords: Restoration, Seedling growth, Nutrients, Herbivory.

Resumo: *Restauração experimental usando mudas de espécies arbóreas é uma estratégia comum para acelerar o processo de sucessão em áreas degradadas usadas pela agricultura em área de Cerrado. O desenvolvimento das mudas em áreas tropicais degradadas é limitado por vários fatores. O objetivo deste estudo foi avaliar o crescimento e sobrevivência de mudas de sete espécies arbóreas nativas usadas para acelerar a revegetação em uma erosão com condições ambientais estressantes. O experimento envolveu blocos fechados e abertos, presença e ausência de fertilização e uso de um adesivo contra herbivoria por formigas (04 tratamentos com 04 repetições). As mudas foram plantadas em Dezembro de 2006 e a coleta de dados sobre diâmetro basal, altura, mortalidade e herbivoria iniciou-se em 24 de Janeiro de 2007 e continuou a cada 03 meses até o final do experimento até 31 de Janeiro de 2009 (totalizando 9 datas de coleta). A sobrevivência das mudas atingiu 38% e a proteção das mudas não influenciou o crescimento, mas as mudas cresceram mais em resposta a adição de fertilizante contendo N, P e K. O uso do adesivo Tanglefoot para impedir o ataque de formiga não demonstrou influencia no crescimento. O uso de cerca reduziu a mortalidade das mudas, mas combinando cerca com o uso do Tanglefoot não apresentou diferença. A disponibilidade de nutrientes limitou o crescimento e sobrevivência das mudas na erosão. O plantio de mudas pode acelerar a sucessão em áreas degradadas de Cerrado desde que supere as limitações que impedem o crescimento e sobrevivência.*

Palavras-chave: *Restauração, Crescimento de Mudas, Nutrientes, Herbivoria.*

1 Introduction

Deforestation and habitat fragmentation are widespread in tropical areas due mainly to human activities with consequent occupation and conversion of the natural landscape for agricultural purposes. These fragmented landscapes are surrounded by human-dominated lands (CELIS; JOSE, 2011; RODRIGUES *et al*., 2011) and the process of deforestation and habitat fragmentation cause losses in biodiversity and environmental services provided by those ecosystems (HOLL; AIDE, 2011; RODRIGUES *et al*., 2011).

The Brazilian cerrado is one of richest savannas in the world and one of the 34 hotspots of biodiversity (MITTERMEIER *et al*., 2004). Its degradation is due to intense production of crops and cattle. If the deforestation process continues at this level it is expected to completely transform this biome by the year 2030 (ANTEZANA, 2008).

In order to slow forest degradation and to conserve native biodiversity in fragmented tropical forests it is important to promote ecological restoration (CHAZDON, 2003). Degraded areas in the tropics are able to regenerate naturally, but the speed at which they recover will depend on the existing vegetation nearby and the land use history (AIDE *et* *al*., 2000; ZIMMERMAN *et al*., 2000; GRISCOM & ASHTON, 2011). Natural recovery in many ecosystems can take decades as succession may be arrested due to low propagule availability and sprouts from roots in the soil, caused by intensive use of the land for agriculture and grazing. Therefore, to accelerate the restoration process and obtain the desired species composition we should consider accelerating succession with methods that use human intervention (HOLL; AIDE, 2011; HOLL *et al*., 2011; CELIS; JOSE, 2011). These interventions could range from removing human disturbances to active restoration efforts, which include planting tree seedlings (HOLL; AIDE, 2011).

The majority of studies on forest recovery and secondary succession have been conducted in moist tropical forests in the neotropics (UHL *et al*., 1988; HOLL, 1998; NEPSTAD *et al*., 1991; PARROTTA *et al*., 1997; AIDE *et al.,* 2000), but a small number of studies have tested methods for restoration of drier forests (FELFILI *et al*., 2005; GRISCOM & ASHTON, 2011; PILON; DURIGAN, 2013). Planting tree seedlings is a common restoration approach used to accelerate tropical forest recovery of degraded areas (HOLL *et al*., 2011; KAGEYAMA; GANDARA, 2000).

The cerrado flora is relatively well known (RATTER *et al*., 2003; RATTER *et al*., 2011; FELFILI; FAGG, 2007; MEWS *et al*., 2012; SILVA; FELFILI, 2012; SOLÓRZANO *et al*., 2012), but little is known about the cultivation of tree species from cerrado vegetation and this inhibits the uptake of ecological restoration approaches (PILON & DURIGAN, 2013). Significant advances in restoration techniques for degraded ecosystems have been achieved all over the world including Brazil. The validated protocols widely replicated in Brazil are based on studies of tropical forest secondary succession; especially studies based on forest gap dynamics (DENSLOW, 1980; WHITMORE, 1984; PILON; DURIGAN, 2013). Some studies have shown that Cerrado can regenerate within 30 years if the soil seed bank and sprouting tree roots from soil are not compromised (PILON; DURIGAN, 2013).

Seedling growth in degraded tropical lands is constrained by various barriers, which include low soil fertility and poor soil structure, harsh microclimatic conditions, and biotic factors such as predation, herbivory, and competition (HOLL, 2002). Most restoration projects in cerrado have failed to achieve their goals because of a lack of knowledge of Cerrado species performance in stressful conditions (CELIS; JOSE, 2011;

PILON; DURIGAN, 2013; FELFILI *et. al*. 2005; PINTO *et al*., 2007). However, recent studies have been performed to search for species that are able to overcome the stressful conditions in degraded areas in the cerrado biome and thus guarantee the success of restoration using native species seedlings (FELFILI *et. al.* 2005; SAMPAIO *et al.,* 2007; PILON; DURIGAN, 2013).

For the planting of seedlings to succeed as a means of restoration, a better understanding of species traits and site characteristics is required (CELIS; JOSE, 2011; HOLL *et al*., 2011). The goal of this study was to evaluate the performance of seedlings of seven native tree species for use in a strategy to accelerate forest recovery in a gully area with stressful environmental conditions. To test this restoration approach, seven tree species that are used for restoration in Brazil were planted in the experimental area (Kageyama & Gandara, 2000). The specific questions asked by this study were:

1. Are seedling growth and survival limited by nutrient availability in degraded Cerrado environments?
2. Are seedling growth and survival limited by leaf-cutter ant herbivory?
3. Does vertebrate herbivory limit establishment of tree seedlings?

2 Methods

2.1 Study site

The area used for this research is located on the right bank of the Araguaia River (17º52'40" - 17º53'40"S; 53º05'40" - 53º07'40"W) in Goias State (Brazil). The experiment area (Figure 1) is close to a gully, which was originally formed in December 1983 as a result of intensive land use for agricultural purposes. The dominant soil in the area is a Typic Quartzipsament (RQa) and the climate is Tropical Humid, with a dry season of 3 to 6 months. The study site is entirely within cerrado vegetation, which is in terms of biodiversity, one of the richest savannas in the world. Local vegetation now is reduced to isolated fragments surrounded by agricultural plantations

2.2 Experimental design

Eight randomized blocks were installed in the area of Chitolina gully from which the soil was displaced after a landslide event; four blocks were fenced and four were unfenced (Figure 1).

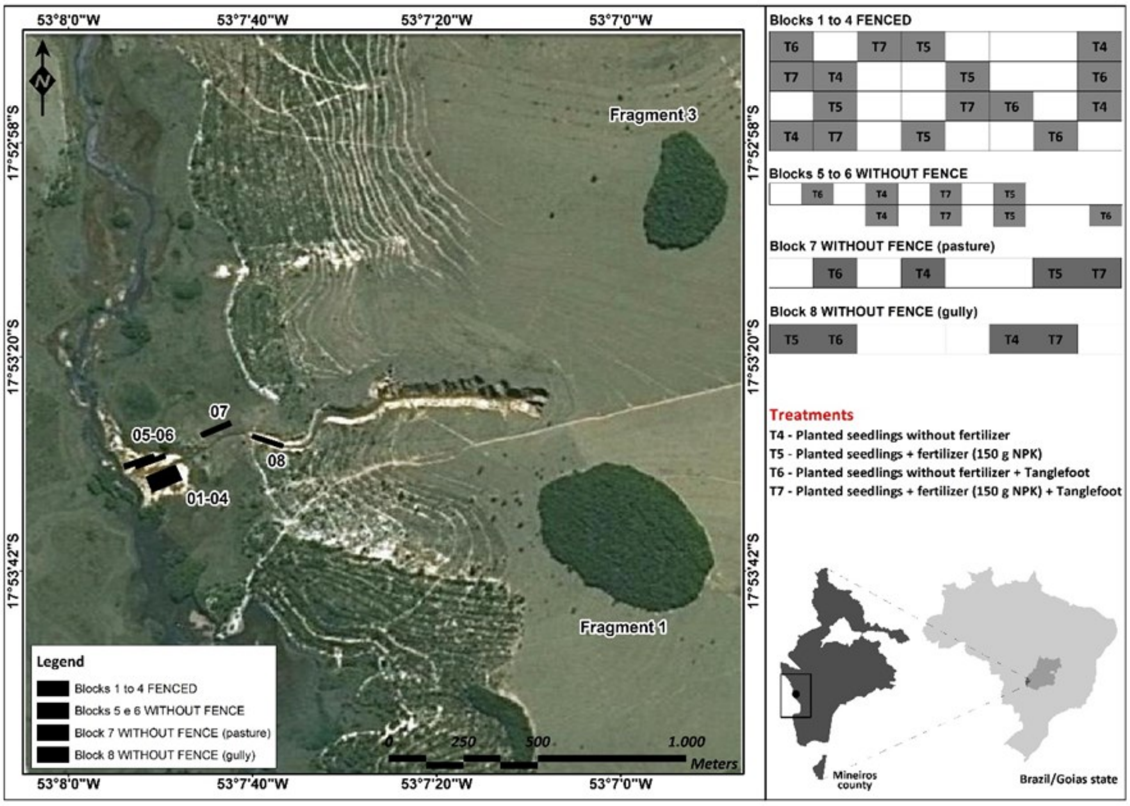

Figure 1. Satellite image of the experimental area, showing the location of the experimental plots. Chitolina gully is the linear feature in the centre. Fragments 1 and 3 are patches of surviving forest within the pasture.

The experiment was established in the field using fenced and unfenced blocks, the presence and absence of fertilization and use of an adhesive for preventing herbivory by ants. Each block had four different treatments as follows: T4 (Control), seedlings of selected species were planted using an arrangement described by Kageyama and Gandara (2000) to monitor seedling growth rate and mortality; T5 (Fertilization – FT), same treatment as Control but with the application of 150g of fertilizer (NPK 5-25-15) to monitor the influence of nutrient availability on growth rate and mortality; T6 (Tanglefoot – TG), same as Control but with the use of the adhesive Tanglefoot to exclude herbivory by ants; T7 (Fertilization + Tanglefoot – FT+TG, same as T5 but with the use of Tanglefoot (a non-drying, sticky compound that forms a barrier against climbing insects) to monitor the interaction of fertilization and herbivory by ants. The installation of the blocks is shown on Figures 1 and the blank plots were used to carry out another experiment.

Limitations on funding, access and availability of suitable land pre-determined the design of the experiment. The area where soil that had been eroded from the gully was deposited was too small to accommodate all the fenced and unfenced blocks in the experiment. In addition, the high cost of fencing prevented to fence each block individually. For these reasons, the optimum alternative design was to fence four blocks together and two unfenced blocks placed within the deposition area of the eroded soil. An additional unfenced block was located in pasture vegetation between the deposition area and the base of the gully (block 7), and a fourth unfenced block (8) was located within the gully (see Fig. 1 for a map showing the distribution of blocks). An optimum design for this experiment would have been to locate an equal number of fenced and unfenced blocks at random locations across the sample area. For the reasons described above this optimum design was not possible, and to mitigate this problem it was adopted not to attempt of interpreting the block effect in this experiment as an effect of fencing.

Subject to this caveat, including fenced blocks permitted to observe the potential influence of small mammals on seed predation and seedling herbivory.

Each block measuring 96 x 12 m was divided into 8 plots of 12 x 12 m. These plots were used to conduct the trials with different treatments and other experiments to determine the constraints on natural re-vegetation of the gully. The fence to exclude vertebrates and small mammals had a mesh of 15 mm x 15 mm and was installed from 30 cm below the soil surface to 1.20 m above-ground.

The seedlings were planted in December 2006. Seedlings of seven species of pioneers and non-pioneers were selected for planting. The selection was based on natural occurrence of the species in the region, availability of seedlings in a nearby nursery and their prior use in restoration projects. The selected pioneer species were *Guazuma ulmifolia* Lam., *Croton urucurana*, and *Jacaranda acutifolia*, and the non-pioneer species were *Enterolobium sp., Luehea candicans Mart., Pseudobombax longiflorum (Mart. & Zucc.) A. Robyns.* and *Inga spp.* Planting followed the arrangement used by Kageyama and Gandara (2000). The pioneer species were planted at a spacing of 2.85 m x 2.85 m. The non-pioneer species were planted in the middle of a group of four seedlings of pioneer species. The pattern of planting followed a sequence of seedlings in a line, always changing the species to avoid having the same species side by side. The non-pioneer species were planted in a line between two lines of pioneer species.

Seedlings used in the experiment came from a nursery, located 120 km away from the experimental site, and they were produced for restoration projects supported by a Non-Governamental Organization (NGO) named OREADES. The seedlings were produced using an organic compost substrate in plastic tubes used for native species. All seedlings were the same age, and had a similar history of production and height (varying from around 20 to 30 cm). The seedlings were removed from the plastic tube and planted with the compost substrate.

Treatments were applied immediately after planting, and after 15 days any seedlings that had died were replaced with new seedlings. Tanglefoot was applied to the stem of seedlings in this treatment using a small paint brush. Fertilizer was placed manually in a circle 20 cm away from the seedling. Collection of data on seedling basal diameter, height, mortality and herbivory started on January 24th 2007 and continued every three months, coinciding with the different seasons of the year, until final data collection on January 31st 2009 (9 measurement dates in total). Digital callipers were used to measure basal diameter and seedling height was measured with a ruler. Dead plants were recorded as "no data". Mortality and herbivory were recorded in separate data sets. Herbivory was recorded as the number of plants observed that had evidence of ant attack.

One year after planting the treatments that received fertilization were treated with another application of fertilizer (150g of fertilizer - NPK 5-25-15). The fertilization coincided with the rainy season. The fertilizer was selected because it is commonly used in restoration projects that involve planting seedlings in the Cerrado region.

2.3 Data analysis

As the unfenced plots were in three different environments, the statistical analysis was carried out by combining all the data for the unfenced plots into a single block and contrasting that with the second, fenced, block. The variables (number of individuals, basal diameter, height, mortality and herbivory) were tested for homogeneity and homoscedasticity using the *car* package for R v 3.0.2 (FOX; WEISBERG, 2011). Percentage mortality and herbivory were log-transformed prior to analysis.

To analyse percentage mortality and herbivory an ANOVA model was constructed that included terms for block (with two blocks, one fenced and one unfenced), treatment (four treatments with four replicates per block), species (*Croton urucurana*, *Enterolobium* sp., *Guazuma ulmifolia*, *Inga* sp., *Jacaranda acutifolia*, *Luehea candicans* and *Pseudobombax longiflorum*) and the interaction between species and treatment. The four treatments were applied to seedlings planted on four 12 x 12 m sub-plots selected at random from 12 x 96 m plots comprising eight contiguous sub-plots (the remaining sub-plots were used for another experiment. Each block represented four plots and therefore four replicates of each treatment. For the fenced block, the four plots were contiguous, while the unfenced block combined four non-contiguous plots as described above (Fig. 1).

As the dependent variables for growth (growth in height and in basal diameter) were measured on the same group of plants at various points in time (nine measurement dates), comparisons between species and treatments on seedling growth (height and basal diameter) were assessed by repeated measures ANOVA, with measurement date (time) as a within-subjects repeated factor, and species and treatment as between-subject factors. By the end of the ninth measurement date, despite starting with four replicates for each treatment in fenced and unfenced blocks, a large number of seedlings had died and there were two replicates that did not have the full set of species represented (one species missing in each replicate). Therefore, in order to achieve a balanced data-set for every treatment at every measurement date the final analysis was conducted on data using the model of two blocks, which contained four treatments, seven species and nine measurement dates. Arithmetic means for height and basal diameter were calculated at each measurement date by species in each treatment, considering fenced and unfenced blocks. To calculate the arithmetic mean, the total sum of each species height and basal diameter in each treatment was divided by the number of individuals alive at that time. Data analyses were carried out using the Repeated Measures procedure available in SPSS Statistics for Windows, Version 22.0 (IBM CORP, 2013).

Changes in seedling height and basal diameter of the surviving individuals were analysed by repeated measures ANOVA with one within subjects factor (measurement date), and two between subjects factors (treatment and species), and the model included a term for the interaction between species and treatment. Because the sphericity assumption was not met (Mauchly's test), the lower-bound correction was used to adjust the degrees of freedom (the most conservative test), which made them smaller and by the reducing of the degrees of freedom, which made the F-ratio more conservative. Pairwise comparisons were performed using a Bonferoni correction when ANOVA results showed significant factor effects. All analyses were carried out using SPSS v.22.0 (IBM CORP., 2013).

Seedling mortality was calculated for each species as the number of dead seedlings at the end of the study period in each plot and treatment, divided by the number of seedlings planted at the start and multiplied by 100 to express mortality as a percentage. Seedling herbivory was calculated for each species by summing the number of individuals attacked throughout the experiment, dividing that total by the number of observations (nine) and multiplying the result by 100 to express herbivory as a percentage. The data analysis for mortality and herbivory were performed using R project v 3.0.2. (R DEVELOPMENT CORE TEAM, 2012)

3 Results

At the start of the experiment 1311 individuals were measured; at the last measurement there were 500 individuals surviving. This means that 38% of the individuals remained alive after two years. A total of 8125 measurements were made from the start to the final date. Values for measured variables (height and basal diameter) by treatment and species were estimated and obtained from SPSS output.

There was no evidence of an effect of block (fence and unfenced) on seedling height, but seedling height differed significantly among treatments (Table 1).

Table 1. Analysis of Variance for seedling height using repeated measures

Transformed Variable:	Average				
Variation source	Type III Sum of Squares	df	Mean Square	F	p(same)
Intercept	58663.99	1	58663.99	310.151	0.000
Block	1.01	1	1.01	0.005	0.942
Treatment	13697.31	3	4565.77	24.139	0.000
Species	42470.10	6	7078.35	37.423	0.000
Treatment * Species	8724.54	18	484.69	2.563	0.013
Error	5106.95	27	189.15		

p<0.05: significant

Comparisons using Bonferoni tests identified that the treatments that showed the highest means for seedling height were Fertilization (FT) and Fertilization+Tanglefoot ((FT+TG), which were not significantly different from each other. Tanglefoot (TG) and Control were not significantly different and they displayed the lowest values of mean seedling height (Figure 2). These results suggest that fertilization enhanced seedling height growth. The application of Tanglefoot had no influence on growth in seedling height.

Mean seedling height was significantly different among species. The species with the highest value was *Enterolobium sp* (Leguminosae) and it was followed by *Inga spp* (Leguminosae) and *Croton urucurana*. These three species were not significantly different and showed the fastest growth rates throughout the period of study (Figure 2). The species that showed the lowest value for mean seedling height was *Jacaranda acutifolia* (Bignoniaceae), but it was not significantly different from *Luehea candicans* and *Pseudobombax longiflorum* and these species showed the lowest mean seedling height at all measurement dates (Figure 2).

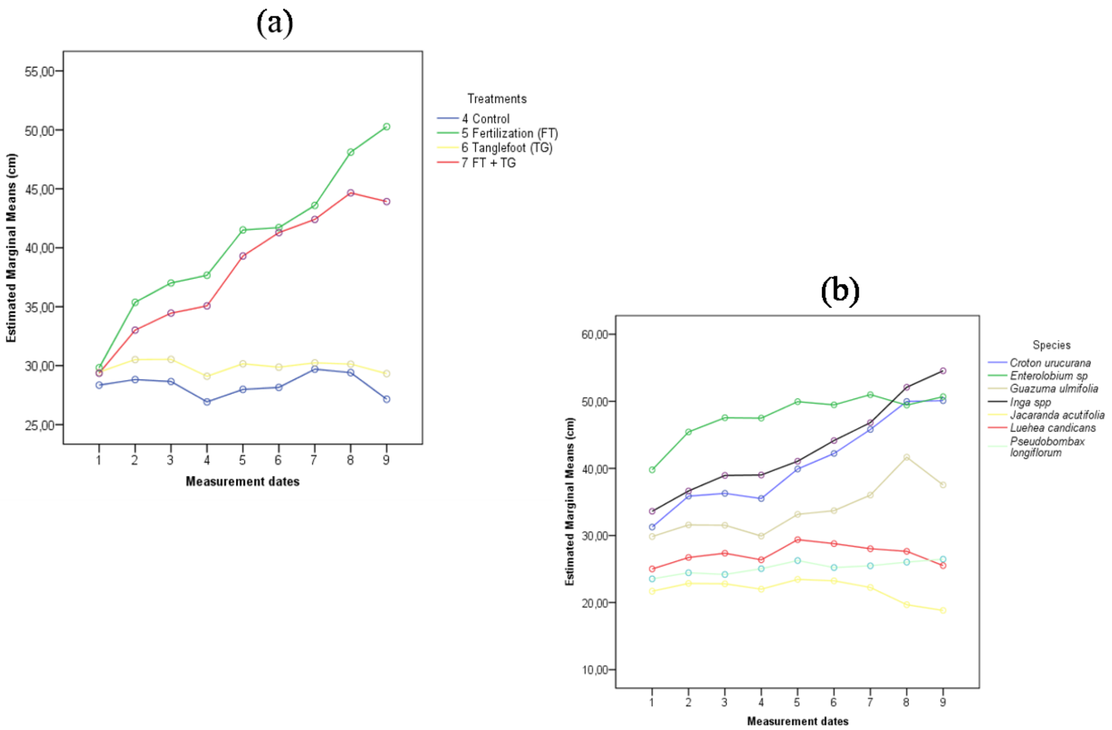

Figure 2. Estimated marginal means (cm) for seedlings height (cm). (a) Different treatments and (b) Different species

Seedlings of *Jacaranda acutifolia* showed no response to fertilization in terms of height growth (Figure 3), while seedlings of *Inga spp* showed the greatest growth response to fertilization and attained the highest mean seedling height at the end of the experiment for treatments involving fertilization (77.42 cm and 72.09). The species that were second and third highest ranking in terms of seedling height were *Enterolobium sp* and *Croton urucurana*. Seedlings of *Jacaranda acutifolia*, *Luehea candicans* and *Pseudobombax* *longiflorum* showed the lowest mean heights for treatments using fertilization (Figure 3) and those of *Jacaranda acutifolia* had the lowest mean height in all treatments and for all measurement dates.

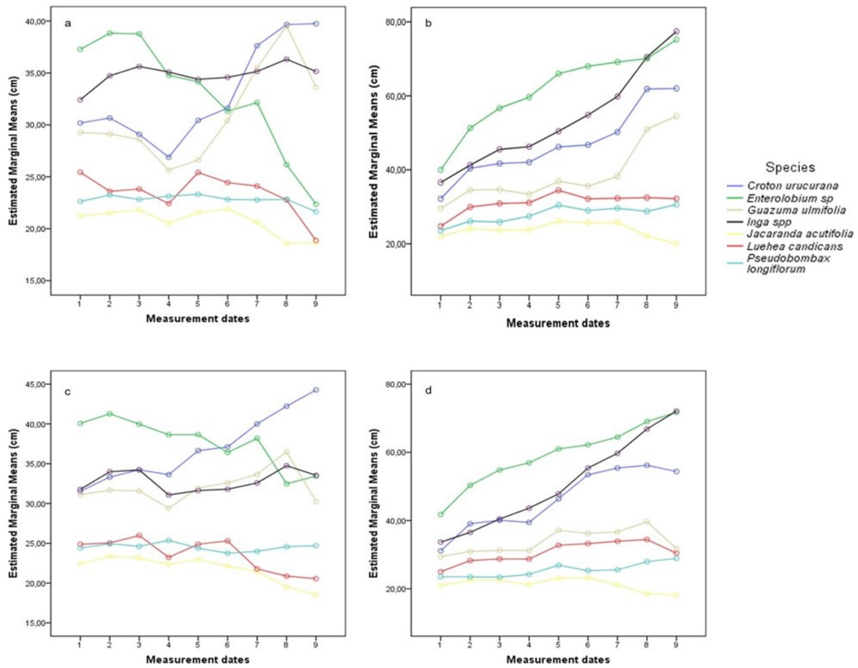

Figure 3. Effects of treatment and species on seedling height (cm) during the whole study period (a) Control; (b) Fertilization (FT); (c) Tanglefoot (TG); (d) Fertilization (FT) + Tanglefoot (TG).

Seedlings of *Inga spp* displayed the highest mean height increment followed by those of *Enterolobium sp*. Seedlings of *Jacaranda acutifolia* had a negative mean height increment when measured across the whole period of study.

According to the Analysis of variance presented in Table 2, there is no evidence of a significant difference between blocks for basal diameter, however mean values of this variable differs significantly among treatments. There was also a significant difference among species in relation to mean of basal diameter, but no significant interaction between treatments and species.

Table 2. Analysis of Variance for seedling basal diameter using repeated measures

Transformed Variable: Average					
Variation source	Type III Sum of Squares	df	Mean Square	F	P(same)
Intercept	2474.75	1	2474.75	162.04	0.000
Block	15.09	1	15.09	0.99	0.329
Treatment	815.39	3	271.79	17.80	0.000
Species	1221.08	6	203.51	13.33	0.000
Treatment * Species	486.82	18	27.05	1.77	0.087
Error	412.35	27	15.27		

$p<0.05$: significant

Pairwise comparisons using Bonferoni tests suggested that treatments FT and FT+TG, which showed the highest means for seedling basal diameter, were not significantly different from each other. Treatment FT showed the highest value for this parameter followed by treatments FT+TG, TG and Control. Mean basal diameter for treatments TG and Control were also not significantly different from each other but they were significantly lower than those for treatments FT and FT+TG (Figure 4).

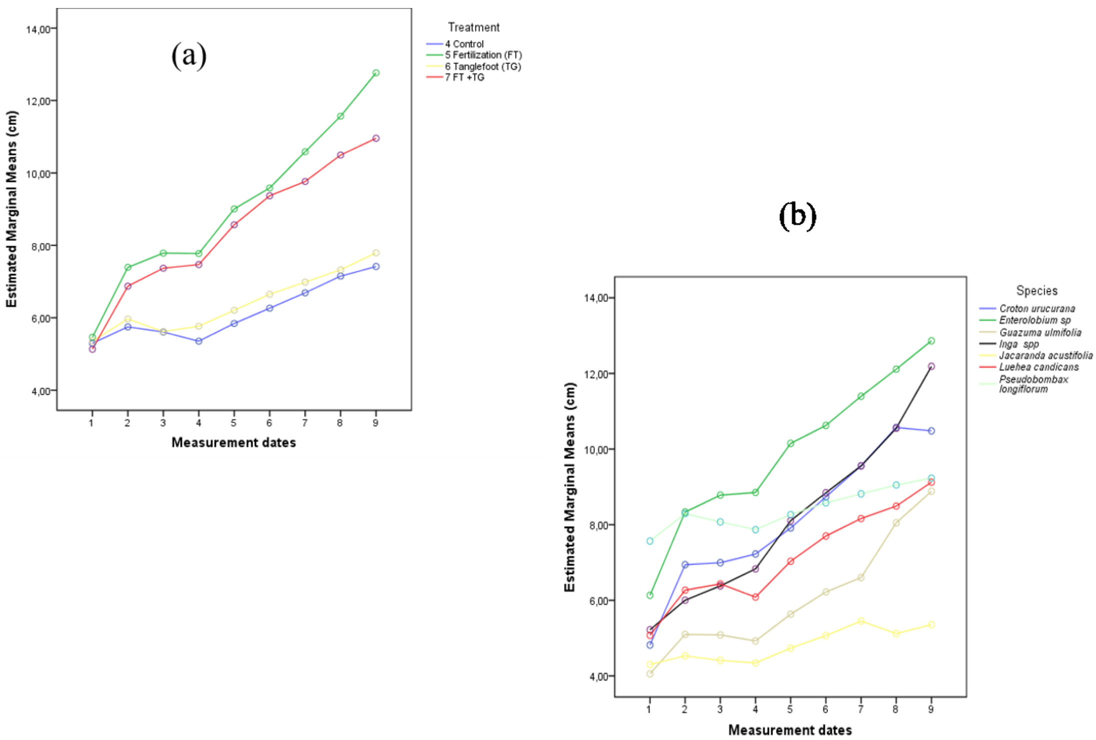

Figure 4. Estimated marginal means (cm) for seedlings basal diameter (cm). (a) Different treatments and (b) Different species

These analyses suggest that Tanglefoot application had no significant effect on growth in seedling basal diameter. The mean for Treatment TG did not differ from the mean for the Control treatment.

Mean basal diameter differed significantly among species. The highest value was for *Enterolobium sp,* which differed from all other species. All species studied showed positive growth in basal diameter throughout the period of study (Figure 4).

Fertilization had a positive influence on basal diameter growth for the different species, an exception was for *Jacaranda acutifolia* (Figure 5). The lowest value for basal diameter mean was for *Jacaranda acutifolia*, which was significantly lower than any other species, apart from *Guazuma ulmifolia*.

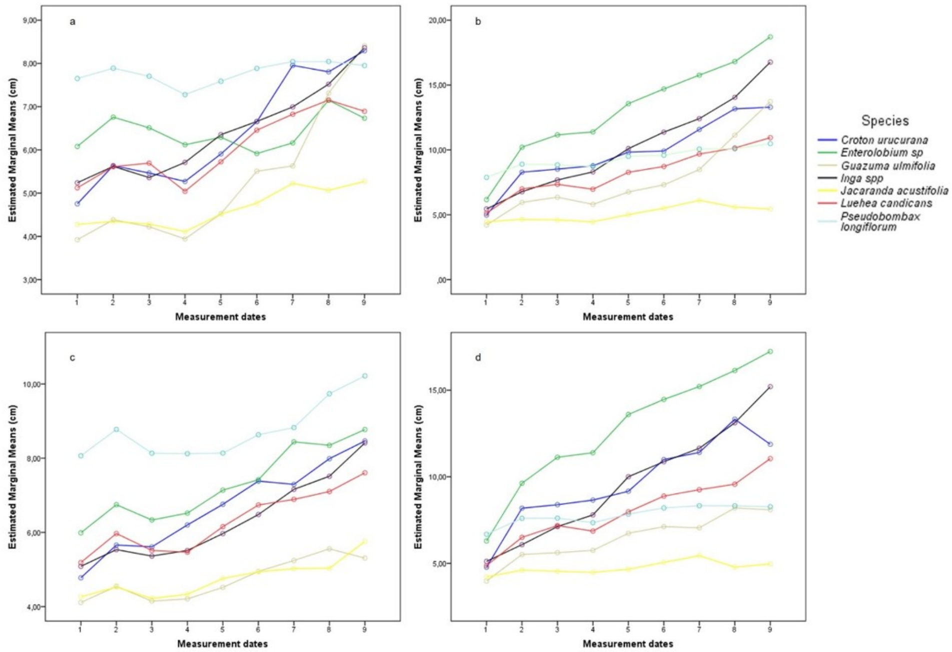

Figure 5. Effects of treatment and species on seedling basal diameter (cm) during the whole study period (a) Control; (b) Fertilization (FT); (c) Tanglefoot (TG); (d) Fertilization (FT) + Tanglefoot (TG).

There was statistically significant difference between fenced and unfenced blocks for seedling mortality (Table 3). The effect of treatments on seedling mortality were marginally non-significant (p = 0.065), but the differences in mortality among species and the interaction between treatment and species were both highly significant.

Table 3. Analysis of Variance for seedling mortality during study period

ANALYSIS OF VARIANCE (ANOVA)					
Variation Source	df	Sum of Squares	Mean Square	F	p(same)
block	1	7454	7454.0	14.74	1.67E-04 ***
treatment	3	3709	1236.2	2.45	0.065
species	6	125516	20919.4	41.37	< 2.2E-16 ***
treat:species	18	43314	2406.4	4.76	1.01E-08 ***
Residuals	195	98597	505.6		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

There was no significant treatment effect on seedling mortality according to the ANOVA, but the fertilization treatments showed lower seedling mortality. The use of the adhesive Tanglefoot did not influence seedling mortality (Figure 6a)

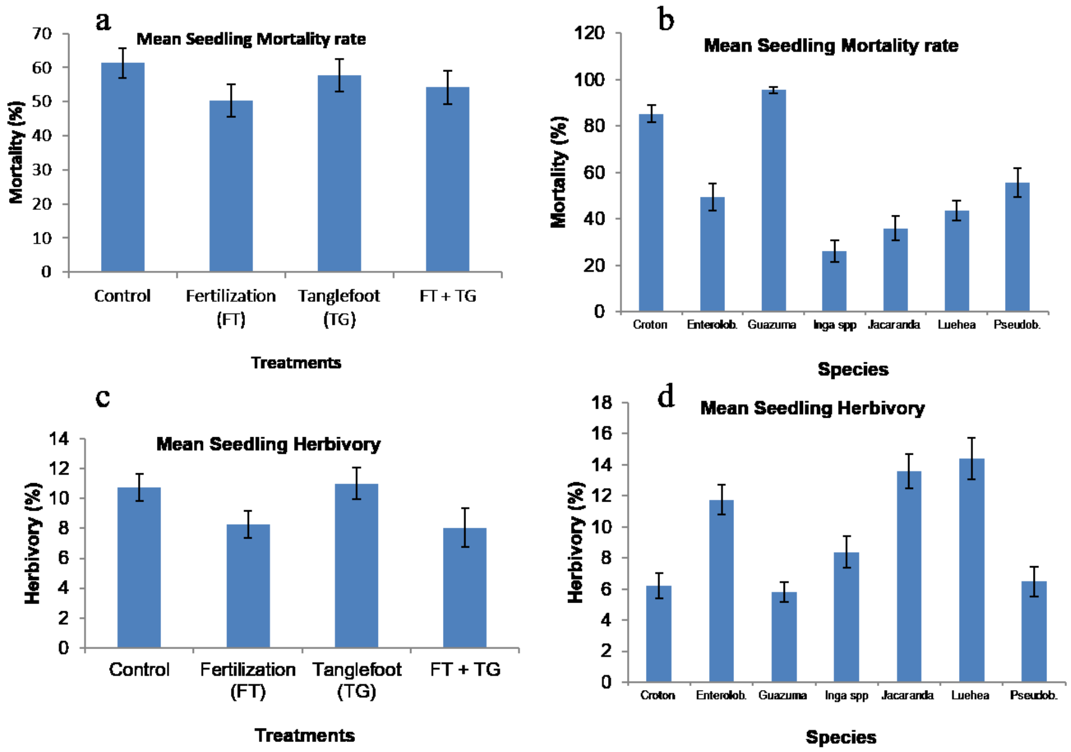

Figure 6. (a) Mean mortality rate for different treatments (b) Mean seedling mortality rate for the seven species used (c) Mean rate of herbivory on seedlings for the four treatments (d) Mean (± SEM) percentage herbivory on seedlings of seven species

There was a significant difference in seedling mortality among species, and a strong interaction between species and treatments on seedling mortality. The lowest mean mortality among species was for *Inga spp* and this was different from all other species in terms of mortality rate. The second ranked species in terms of mortality was *Jacaranda* *acutifolia,* followed by *Pseudobombax longiflorum, Luehea candicans* and *Entorolobium sp*, which did not differ from each other. The highest mortality rates were displayed by *Guazuma ulmifolia* and *Croton urucurana*, and the means of these two species are not significantly different from each other (Figure 6b).

The response of seedling mortality to fertilization was variable among species. Seedlings of *Jacaranda acutifolia* and *Croton urucurana* showed higher mortality rates in treatments FT and FT+TG, which indicate that they were sensitive to fertilization. The mortality rates for these species in the two unfertilized treatments were lower. By contrast seedlings of *Enterolobium sp.*, *Inga spp.* and *Pseudobombax longiflorum* showed opposite results, where the highest mortality rates were found in treatments without fertilization (Control and TG) and the lowest rates in treatments with fertilization (FT and FT + TG). Seedlings of *Guazuma ulmifolia* and *Luehea candicans* showed no marked response to fertilization.

There was no significant difference between the fenced and unfenced blocks for herbivory, although the mean for the fenced block was lower than that for the unfenced block. There were statistically significant differences in herbivory among treatments and species, but no significant interaction between these factors (Table 4).

Table 4. Analysis of Variance for seedling herbivory during the study period

ANALYSIS OF VARIANCE (ANOVA)

Variation Source	df	Sums of Squares	Mean Square	F	p(same)
block	1	55.5	55.49	1.76	0.187
treatment	3	419.6	139.85	4.43	0.005 **
species	6	2573.0	428.84	13.58	7.4E-13 ***
treatment:species	18	204.0	11.33	0.36	0.99
Residuals	195	6159.5	31.59		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Pairwise Tukey’s tests suggested that treatments Control and TG, which displayed the highest mean herbivory values, were not significantly different. The lowest mean rate of seedling herbivory occurred in the fertilization treatments (FT and FT+TG), which were not significantly different from one another (Figure 6c).

Pairwise Tukey tests identified that herbivory on seedlings of *Luehea candicans* and *Jacaranda acutifolia*, which displayed values across species, were not significantly different. These two species had distinctly higher rates of herbivory than seedlings of *Enterolobium* sp., which was higher than the four remaining species (Figure 6d).

4 Discussion

4.1 Seedling survival and growth rate

Of the original 1311 seedlings that were planted in this experiment, only 500 survived across all species after 24 months (38%), which is consistent with the value of 35 % survival of seedling transplants obtained by Lazarini *et al*. (2001) over a 12 month period using other native species in cerrado vegetation in the Federal District area of Brazil. Annual mortality rate for all species was around 31.0 % yr^{-1}, which is consistent to the value obtained by Antezana (2008) working with native cerrado tree species (annual mortality of 33.33%) in a restoration experiment conducted at Planaltina in the Federal District of Brazil. Sampaio *et al*. (2007) working with 18 native cerrado species planted, with different treatments, in abandoned pasture in central Brazil (Goias State) found an overall mortality rate of 26.3 % over a 14 month study period. Pinto *et al*. (2007) found a higher value for seedling mortality in abandoned pasture (43%) in regional cerrado at Unai (Minas Gerais State) over a 14 month study period.

Mortality rates were species-specific, which suggests that the ability to survive in a range of stressful conditions varied from one species to another. The lowest value for mean mortality was shown by *Inga spp* (26.0%). Similarly, Holl *et al*. (2011) found that survival rates were higher for *Inga edulis* than for 3 other species in a plantation experiment in a former agricultural area in Costa Rica, which corroborates the findings of the present study. Silva and Correia (2008) registered high survival values (93.3%) for *Inga marginata* in a mining restoration study in cerrado vegetation. In this study, the highest mortality rates were presented by the pioneer species *Guazuma ulmifolia* and *Croton urucurana*. The results of this study for *Guazuma ulmifolia* therefore contrast with the results reported by Sampaio *et al*. (2007) who found a low value for seedling mortality over 14 months in a plantation trial in abandoned pasture in central Brazil. These two species (*Guazuma ulmifolia* and *Croton urucurana*) are very attractive to leaf cutting ants, which may be the explanation for their low survival in the field.

Pioneers species tend to be more sensitive to leaf cutter ants attack, because of their strategy of fast growing and rapid colonization. However, in harsh environmental conditions they may display a low survival rate because of a reduced capacity to tolerate or resist leaf cutter ants attack. Non-pioneers grow slowly, display high survival under forest understorey conditions, and may be more resistant and less attractive to ants than pioneers.

The species in this experiment differed in their seedling growth rates. Seedlings of *Enterolobium* sp and *Inga* spp grew most quickly, which is consistent with other studies that have used species from these genera. For example, Pilon and Durigan (2013) screened 106 cerrado species to establish criteria for identifying priority species for restoration of cerrado vegetation and reported high values of annual height growth for *Enterolobium gummiferum* (60 cm) and *Inga laurina* (30 cm). As a result, these species were given high attribute values for their suitability for use in restoration (above 78 – on a scale from 0 to 100). In addition, Holl *et al*. (2011) found that *Inga edulis* had the highest growth rate in height among four tree species examined for their suitability for use in restoration in Costa Rica. Silva and Correia (2008) found that *Inga marginata* was one of three species, out of six, with the highest height growth rates in a mining area restoration experiment in the Federal District of central Brazil.

4.2 Effects of excluding of vertebrate herbivores

Exclusion of vertebrate herbivores using fences did not influence seedling growth rates in height or diameter, but survival was greater in fenced than unfenced blocks. This result has to be interpreted with caution because the fenced and unfenced blocks were not distributed independently and at random across the study area of degraded cerrado used in this experiment. However it is noticeable that Antezana (2008) found higher mortality of cerrado species in unfenced (34.0 %) than fenced (26.3 %) plots. My study site was frequented by the Tapir (*Tapirus terrestris* Linnaeus), one of the biggest herbivores in Brazil, which may have consumed entire seedlings in unfenced block, resulting in them being recorded as dead. This herbivore is known to consume tree seedlings, as found in a study conducted in Atlantic forest in Brazil (BACHAND *et al*., 2009). The fence in this study did not affect growth rates of height or basal diameter, as these variables were measured only for individuals that were alive. Higher survival following exclusion of vertebrates is consistent with findings of studies conducted in neotropical sites

(GRISCOM *et al*., 2009; HOLL; QUIROS-NIETZEN, 1999; WILLIAMS-LINERA *et al*., 1998) but contrasts with the conclusions of Gunaratne *et al*. (2011) who used much smaller enclosures around individual seedlings planted into a human-derived grassland in Sri Lanka.

4.3 Effects of nutrient addition on seedling growth and survival

Seedling growth increased in response to the addition of fertilizer containing the nutrients Nitrogen, Phosphorus and Potassium (NPK). Nutrient availability commonly limits tree growth or forest productivity in tropical ecosystems. For example, addition of NPK provided evidence that nutrient deficiency limited plant growth in an experiment conducted in a tropical forest in Panama (WRIGHT *et al*., 2011). The same authors found also that addition of K decreased fine-root biomass and they pointed out that limitation by the availability of K might deserve greater attention. Addition of nutrients (NPK) in a secondary savanna in Venezuela resulted in higher aboveground biomass for all vegetation (BARGER *et al*., 2002). A review of the effects of soil nutrients in tropical dry forest in India suggests that N and P application increases the growth of seedlings of woody species (KHURANA; SINGH, 2001). Tropical forest tree seedlings have been shown to increase growth in response to addition of N, P and Mg in pots (BURSLEM *et al*., 1995). In the present study, seedlings of *Enterolobium* sp and *Inga* spp showed a greater increase in height in response fertilization (59.7 and 53.3 % respectively) with or without tanglefoot than the other species (range 6.7 to 28.2 %), and seedlings of *Jacaranda acutifolia* had negative increment in height in response to fertilization. Pilon and Durigan (2013) working with two different species of *Jacaranda* found that they were generally unsuitable for use in cerrado restoration projects (on the scale from 0 to 100 they scored 67, from a group of 106 different species, 68 species had better performance than these two species).

Nutrient addition increased seedling survival, which is consistent with some studies carried out in tropical forests. In this study seedlings of all tree species exhibited an overall mean mortality of 56% a value comparable to results reported for tropical forest in Yucatan (CECCON; SANCHES; CAMPO, 2004), where mortality rates ranged from 54–63% after 24 months period. Our findings show that fertilization can lead to seedling survival increasing from 8.5 to 28.3% compared with controls. For this study, it is suggested that nutrient availability is a key factor influencing seedling survival. This finding is similar to that observed in tropical forest studies (SALINAS-PEBA *et al*., 2013; CECCON *et al*., 2006). Working in tropical dry forest in Mexico, Ceccon; Sanches & Campo (2004) also found that P addition over two growing seasons led to increased seedling survival time in secondary forest. By contrast, seedlings of *Jacaranda acutifolia* and *Croton urucurana* in the present study had higher values of seedling mortality in the treatments that used fertilizer. The *Jacaranda* suffered more severe mortality in response to nutrient addition than *Croton*, which may be related to interactions with herbivores. In this study seedlings planted in treatments involving addition of NPK displayed lower rates of herbivory (8.03 – 8.28 %) than the unfertilized controls (10.75 – 11.01 %). This result contrasts with the findings of Santiago *et al*. (2012), where addition of P and K, but not addition of N, increased herbivory on leaves. In my study, herbivory rates were variable among species; the highest rate was observed for seedlings of *Luehea candicans*, and the lowest was observed for those of *Guazuma ulmifolia.* In general, an increased availability of nutrients may enhance the tolerance of seedlings to herbivores, which may explain the lower frequency of herbivory on fertilized seedlings.

4.4 Effects of Tanglefoot on seedling growth and survival

The use of Tanglefoot had no influence on rates of growth or survival. The treatment using this adhesive led to no significant difference when compared to the control. This may be explained by the fact that the soil at the study site is very sandy (defined as a Typic Quartzipsament (RQa)) and it lacks a vegetation cover. Therefore during the first rainfall event after the installation of the experiment, sand particles adhered to the stems of all seedlings, and this enabled ants to climb over the Tanglefoot layer. A study conducted in Costa Rica using seedlings of *Stryphnondendron microstachium* also found that Tanglefoot had no effect on herbivores, and that plants with Tanglefoot had significantly more herbivore damage and grew less than did control plants (LA FUENTE; MARQUIS, 1999). On the other hand, Kelly (1986) had success in an ant exclusion experiment when applying Tanglefoot to *Cassia fasciculata.*

5 Conclusions

Direct planting of seedlings of native trees may accelerate succession in degraded cerrado lands subject to interventions that overcome constraints on seedling growth and survival. In my experiment, nutrient availability was the single most important factor limiting seedling growth and survival in the Chitolina gully area, although some species responded negatively to nutrient addition and may be unsuitable for use in restoration projects. Therefore, my results support the widespread use of fertilizers to enhance seedling establishment in restoration projects and careful species choice to avoid species that may respond negatively to nutrient addition (CECCON *et al*., 2006; SALINAS-PEBA *et al*., 2013).

Herbivory due leaf-cutter ants (Atta sp and Acromyrmex sp) may limit seedling growth and survival, although in this experiment the use of Tanglefoot was not successful at preventing access to seedling canopies by leaf cutter ants. In addition to ant-induced herbivory, there is circumstantial evidence that vertebrate herbivores reduced tree seedling survival in the Chitolina gully, and it is likely that fencing will be required in any large-scale implementation of plantations for ecosystem restoration. However, further research is still required to identify species that are resilient to shortage of nutrients, herbivory and the harsh microclimatic conditions that are typical of degraded cerrado vegetation.

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