2010-04-08

Seed Dynamics and Seedling Establishment of Woody Species in the Tropical Savannas of Central Brazil (Cerrado)

Ana A. Salazar Parra
University of Miami, ana@bio.miami.edu

Recommended Citation
Salazar Parra, Ana A., "Seed Dynamics and Seedling Establishment of Woody Species in the Tropical Savannas of Central Brazil (Cerrado)" (2010). *Open Access Dissertations*. 371.
https://scholarlyrepository.miami.edu/oa_dissertations/371

UNIVERSITY OF MIAMI

SEED DYNAMICS AND SEEDLING ESTABLISHMENT OF WOODY SPECIES IN THE TROPICAL SAVANNAS OF CENTRAL BRAZIL (CERRADO)

By

Ana A. Salazar Parra

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

May 2010

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

SEED DYNAMICS AND SEEDLING ESTABLISHMENT OF WOODY SPECIES IN THE TROPICAL SAVANNAS OF CENTRAL BRAZIL (CERRADO)

Ana A. Salazar Parra

Approved:

_________________ Guillermo Goldstein, Ph.D. Professor of Biology	_________________ Terri A. Scandura, Ph.D. Dean of the Graduate School
_________________ David Janos, Ph.D. Professor of Biology	_________________ Leonel Sternberg, Ph.D. Professor of Biology

____
Carol C. Baskin, Ph.D.
Professor of Biology and of Plant and Soil Sciences
University of Kentucky

SALAZAR PARRA, ANA A. (Ph.D., Biology)
Seed Dynamics and Seedling Establishment (May 2010)
of Woody Species in the Tropical Savannas
of Central Brazil (Cerrado).

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor Guillermo Goldstein.
No. of pages in text. (180)

Studies of seed dynamics and seedling establishment at the community level in savanna ecosystems are scant, particularly in the tropical savannas of Central Brazil. The Brazilian savannas (cerrado) have the largest diversity of plant species among Neotropical savannas. Cerrado vegetation exhibits consistent changes in tree density and tree size along shallow topographic gradients. Vegetation types differ from closed savannas with high tree density, and a nearly continuous canopy cover in the uppermost portions, to open savannas with scattered short trees in the low portions of the topographic gradients. Whether or not dynamics of seeds and seedlings of woody species are consistent with variations in tree density and tree canopy cover across the cerrado landscape has not been determined, but could potentially influence such spatial patterns of tree density and canopy cover.

The main objective of this study was to evaluate seed dynamics (i.e., seed rain, soil seed banks, and seed characteristics) and seedling establishment of woody species in three major cerrado vegetation types (closed, intermediate and open savannas) which differ in tree canopy cover and tree height. These vegetation types are located along topographic gradients of approximately 30 m in elevation and 1000 m in length.

I conducted field work from June 2005 to August 2008 at the Instituto Brasileiro de Geografia e Estatística (IBGE) reserve, a field experimental station located 35 Km south of Brasilia, Brazil (15˚ 56' S, 47˚ 63' W, altitude 1100 m). I established permanent plots in each of the three vegetation types to assess seed rain, seed limitation, seed predation, and seed removal. I also quantified density and composition of the soil seed bank in each vegetation type and studied seed characteristics (i.e., kind of dormancy, moisture content, longevity) of 14 common cerrado woody species. In the field, I quantified establishment of woody seedlings in fire-protected savannas as well as in accidentally burned and frequently burned savannas. In the greenhouse I performed experiments to evaluate the effect of litter cover and light level on seedling emergence of 9 common cerrado woody species. Finally, I evaluated the effect of pulses of heat on seed germination of 5 common cerrado woody species under controlled conditions.

The overall results of this study show that regardless of tree canopy cover, cerrado vegetation types are limited in seeds and seedlings of woody species, but these limitations are higher in open than in closed savannas. Most woody species do not form persistent soil seed banks along cerrado vegetation types because their seeds are short-lived, predated and quickly removed from the ground. In addition, dispersal season and dormancy appear to control timing of germination at the onset of the rainy season to ensure survival of seedlings in the field. In the absence of fire, establishment of woody seedlings is higher in closed than in open savannas because canopy cover mitigates the stressful environmental conditions of open savannas and thus facilitates establishment of woody seedlings. As a result, closed savannas are likely to maintain higher density of woody elements over time than open savannas. In burned savannas, however, species

composition of woody and herbaceous seedlings is significantly affected because establishment of seedlings of woody and herbaceous species decreases but vegetative reproduction increases. This dissertation study indicates that interactions of early life-history stages of woody species with environmental and disturbance factors appear to play a more significant role in maintaining woody cover variations along cerrado topographic gradients than previously recognized.

Dedication

I would like to dedicate this dissertation to my family, my husband and my father's memory.

My family gave me the strength and resilience to complete this dissertation despite the difficulties.

My husband assisted me with lab and field work. His unconditional love, daily support, encouragement, and blind faith helped me complete this dissertation.

My father taught me the importance of discipline to achieve major goals.

Acknowledgments

I am grateful to my advisor Dr. Guillermo Goldstein for his guidance, time, support, and encouragement throughout all my years as a graduate student at the University of Miami (UM). I am also grateful to my committee members Drs. David Janos, Leonel Sternberg and Carol C. Baskin, for all their help, comments and suggestions on how to improve this dissertation. I also thank my previous advisors in Colombia, Claudia Ramírez Sandoval and Maria Susana Carrizosa, who not only got me interested in seed biology and taught me some techniques I was able to use in this dissertation, but also encouraged me to continue a career in science.

I am very grateful to my husband Daniel Ramírez for all his support throughout all my years as a graduate student. This dissertation is the result of a team-work effort. He not only assisted me with home duties, field and laboratory work, but also gave me a lot of help in improving my English writing. I couldn't have had a better field assistant and a better partner. I will be forever grateful for all his support, help and love. I am extremely grateful to my mother Elena Parra Lleras, my uncle Ernesto Parra Lleras, and my aunt Gloria de Parra for their unconditional support, patience and love. I also thank my brothers Julio, Bernardo and Mario for their encouragement and good cheer all along.

I greatly thank the staff of The Reserva Ecológica do IBGE for their logistic support and the IBGE herbarium staff, in particular Maria Aparecida da Silva for helping me to identify plant specimens. I thank Drs. Augusto C. Franco and M. Haridasan for their great help and support that allowed me to carry out field work in Brazil.

I am thankful to Randol Villalobos, Catarina Cartaxo, Mariana Saraceno, and Marina Scallon not only for assisting me in the field, but also for their friendship. I thank Randol Villalobos, Lucero Sevillano, Floria Mora-Kepfer, Catalina Aristizábal, Eric Manzane, David Matlaga, Tanya Hawley, Frans Juola, Naveen Wijesena, Bhagya Janananda, Robert McEderry, and Amartya Saha for their friendship and support at the University of Miami. I am also extremely grateful to Simone Cappellari, Imma Oliveras, Sybil Gotsch and Erica Geiger for their friendship, help and support in Brazil.

I thank Rafael La Rota, Ana Cifra, Maria Hernandez, and Luis Parra for their support, encouragement and friendship through these years. They have been like my family in Miami since my arrival.

I also thank each of the members of the “Mycorrhiza Discussion Group for all of their comments on my dissertation, and for giving me great feedback on how to improve my dissertation seminar. I am very thankful to Orou Gaoue, David Matlaga, and Lucero Sevillano for providing statistical guidance.

Finally, I thank the National Science Foundation (Biocomplexity project), in particular its principal investigator, Dr. Fernando Miralles-Wilhelm, for allowing my participation in his project and for funding a large part of my field work. I also thank UM Center for Latin American Studies, UM Jerry Curtis Scholarship, UM Kushlan- Frohring Graduate Research Support Fund, and UM College of Arts and Sciences for funding my work.

TABLE OF CONTENTS

Page

List of Tables

List of Figures

Chapter 1

Introduction

Seed dispersal, seed germination, and seedling establishment are crucial processes in plant population dynamics because they largely influence the distribution and abundance of plant species. At local scales, successful plant establishment has to overcome two successive bottlenecks: (1) seed limitation, which is the absence of recruitment because of limited seed supply, and (2) establishment limitation, which is the absence of recruitment because of limited availability of suitable sites for a particular seed to germinate and establish (Harper 1977; Eriksson and Ehrlén 1992; Clark et al., 1998; Muller-Landau et al., 2002).

Studies of seed dispersal, seed germination, and seedling establishment at the community level in savanna ecosystems are scant. Savannas are mainly tropical ecosystems that occupy an eighth of the global land surface and support a substantial proportion of the human population in tropical regions (Scholes and Archer 1997). Savanna ecosystems are characterized by the co-dominance of two contrasting plant life forms: trees and grasses (Sankaran et al., 2004). Although the relative abundance of these life forms varies across savanna types, they typically compose communities with a continuous herbaceous layer and a discontinuous stratum of trees (Sarmiento 1984).

Despite their recognized importance to human welfare and economy, the origin, nature and dynamics of savannas are poorly understood (Jeltsch et al., 1998). In particular, the mechanisms allowing trees and grasses to coexist, and the factors determining the relative proportions of woody and herbaceous components across different savanna types remain unclear (Scholes and Archer 1997, Bond 2008).

Understanding the coexistence between grasses and woody plants in savannas should include information on the early stages of the plant life cycle such as seed dispersal, seed germination, and seedling establishment.

The tropical savannas of central Brazil (cerrado) cover 2.5 million km^2, nearly 23% the area of the country (Silva et al., 2006). The Brazilian cerrado has a unique fauna and the largest diversity of all savanna floras in the world (Oliveira-Filho and Ratter 2002). Cerrado vegetation is very rich in endemic woody species; more than 500 species of trees and large shrubs grow in the region, and individual sites may contain up to 70 or more tree species per hectare, with a similar number of shrub species (Franco 2002; Haridasan 2008). Cerrado vegetation types differ in tree density, tree size (DBH) and canopy height. They occupy different portions along shallow altitudinal gradients (approx 30 m in elevation, and a few kilometers in length) from closed savannas (cerrado denso) with high tree density and a nearly continuous canopy cover in the uppermost portions, to open savannas (campo sujo) with scattered short trees in the low portions of the topographic gradients (Eiten 2001). Other savanna types with intermediate canopy cover (cerrado sensu stricto) occupy intermediate topographic positions. The herbaceous-grass layer cover follows the opposite trend of woody species cover (Eiten 2001).

Cerrado plant species exhibit variations in flower and fruit production that are apparently determined by seasonal variations in precipitation and humidity (Lenza and Klink 2006). Studies have reported seasonal variations in seed dispersal of herbaceous and woody species with a peak during the late dry and early wet seasons (Gouveia and Felfili 1998; Lenza and Klink 2006). Thus, seed dispersal appears to be an important mechanism controlling timing of germination of cerrado species. However, seeds of many cerrado trees are dormant suggesting that dormancy could also control timing of germination by ensuring subsequent growth and survival of seedlings. Little is known about characteristics of seeds of woody species in the cerrado and whether or not they form persistent soil seed banks along cerrado vegetation types. How well the soil seed bank composition reflects the species composition of the standing vegetation also is unclear.

Environmental conditions in the cerrado are stressful for the survival and growth of seedlings because of the five-month dry season (Hoffmann 2000), limited availability of soil nutrients (Haridasan 2008), and frequent burning that occurs at 1-5 year intervals, generally during the dry season (Coutinho 1990; Moreira 2000). Other environmental factors such as light availability and leaf litter cover also could highly constrain the quality and quantity of sites for emergence and establishment of woody seedlings in the cerrado. However, these factors have been poorly studied along different cerrado vegetation types.

Studies on early stages of the life cycle of woody plants at a community and landscape level in the Brazilian cerrado are scarce. In particular, it is not clear whether seed dispersal, seed germination, and seedling establishment of woody species differ among cerrado vegetation types and which factors limit seedling establishment the most. Whether or not dynamics of seeds and seedlings of woody species are consistent with variations in tree density across the cerrado landscape has not been determined, but could potentially influence such patterns of tree density.

In this dissertation research, I evaluated seed dynamics (seed rain, soil seed banks, and seed characteristics) and seedling establishment of woody species in three major cerrado vegetation types that differ in tree canopy cover and tree height. These vegetation types are located along topographic gradients of approximately 30 m in elevation and 1000 m in length.

I conducted field work from June 2005 to August 2008 at the Instituto Brasileiro de Geografia e Estatística (IBGE) reserve, a field experimental station located 35 Km south of Brasilia, Brazil (15˚ 56' S, 47˚ 63' W, altitude 1100 m). Mean monthly temperatures range from 19˚C to 23˚C. Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August often are completely rainless. Average relative humidity during the rainy season is about 80% and drops to 55% during the dry season when daily minimum relative humidity may reach values as low as 10%. Fires are common during the dry season, particularly from August to September, when low relative humidity and dry grass-biomass increase the risk of fire propagation. The soils are very deep, well-drained, red or yellow clay-rich oxisols; structurally strong but nutrient poor. The IBGE reserve contains all major vegetation types from closed to very open savannas.

Background

Seed limitation in plant communities

Growing realization that seeds often may be in short supply has led to increased interest in understanding life history stages where recruitment limitation can occur, including seed arrival at the ground, seed predation, seed germination, and early seedling survival (Clark et al 1998). Seed production and dispersal are critically important processes in population dynamics, precisely because not all sites suitable for a given species are reached by its seeds. The failure of seeds to arrive at all suitable sites limits population growth rates and abundances, a phenomenon called seed limitation (Eriksson and Ehrlen 1992, Turnbull et al., 2000). Seed limitation has important consequences for population and community dynamics and for species diversity at multiple scales (Hurt and Pacala 1995). Seed limitation can arise from limited seed numbers and/or limited dispersal of available seeds. A way to assess seed limitation is to measure patterns of seed rain in the field to determine the proportion of sites that are reached by the seeds. If there are no seeds, there cannot be subsequent seedlings or adults, regardless of establishment conditions. Thus, the proportion of all sites at which seeds do not arrive is a measure of fundamental seed limitation (limitation measured as if no other factors were limiting). Because seed limitation arises from both limited numbers of seeds and limited distribution of available seeds, fundamental seed limitation can be decomposed into source limitation, which is the failure of seeds to reach sites due simply to insufficient seed numbers, and dispersal limitation, which is limitation due to non uniform distribution of seeds among sites (Clark et al., 1998).

In contrast to temperate and tropical forest communities, the role of local-scale seed limitation in tropical savanna communities is less clear. Although reproductive phenology and seed dispersal syndromes have been described for plant communities in the Brazilian cerrados (Mantovani and Martins 1988; Batalha et al., 1997; Oliveira and Gibbs 2002; Batalha and Martins 2004; Gottsberger and Silberbauer- Gottsberger 2006), few studies have evaluated seasonal variation of seed rain (e.g. Souza 1993; Marimon and Felfilli 2006), and no studies have estimated the degree of seed limitation among cerrado vegetation types to determine if seed input may limit the potential recruitment in these tropical savanna communities.

Post-dispersal processes such as seed predation, removal, and seed viability loss may highly constrain recruitment in populations that are seed-limited by further reducing the number of seeds available for germination (Fenner and Thompson 2005; Campbell and Clarke 2006). Such processes may critically affect plant reproductive success and ultimately affect species distribution patterns. These processes, however, have not been studied simultaneously in communities of cerrado woody species.

Seed germination and dormancy

Seed germination incorporates all the events that begin with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black 1994). Most seeds require water, oxygen and a suitable temperature for germination. A dormant seed (or other germination unit) is one that does not have the capacity to germinate in a specified period of time under otherwise favorable conditions of temperature, moisture, and light, whereas nondormant seeds will germinate over the widest range of conditions possible for the genotype (Baskin and Baskin 2005).

Five classes of dormancy are recognized: physiological, morphological, morphophysiological, physical, and combinational (physical + physiological) (Baskin and Baskin 1998). Seeds with *physiological dormancy* do not germinate because the embryo has a physiological “problem” that results in low growth potential of the embryo, but as dormancy break occurs, growth potential increases to the point that germination is possible. *Morphological dormancy* is due to the presence of a small or underdeveloped embryo that must grow to a species-specific critical length before radicle emergence is possible. Seeds with *morphophysiological dormancy* have underdeveloped embryos that are physiologically dormant; thus, germination does not occur until physiological dormancy has been broken and embryos have grown. *Physical dormancy* is caused by one or more water-impermeable palisade layers in the seed (or fruit) coat, and dormancy break involves disruption of a specialized area of the seed (e.g. lens in Fabaceae) or fruit coat, thereby creating an opening, which is the site of water entry. Seeds with *combinational dormancy* have impermeable seed (or fruit) coats plus physiologically dormant embryos. Thus, in order for the seed to germinate the coat has to become permeable to water and the physiological dormancy of the embryo must be broken.

The crucial function of dormancy is to prevent germination when the probability of survival and growth of the seedlings is low (Fenner and Thompson 2005). Considerable diversity in the kinds of seed dormancy exists among plants (Baskin and Baskin 2004) and habitats. In tropical savanna ecosystems, few studies have examined the kind of seed dormancy (or lack of dormancy) of woody species in relation to seed characteristics such as seed moisture content, seed longevity, and dispersal season at the community level.

Soil seed banks

A reserve of viable, un-germinated seeds in a habitat is called a seed bank (Baskin and Baskin 1998). Soil seed banks play an important role in the long-term survival of individuals (Simpson et al., 1989; Baskin and Baskin 1998) as well as in the coexistence of plant species at a particular site because they constitute a source of propagules for recruitment after disturbances (Dalling and Hubbell 2002; Williams et al., 2005), and an alternative source of propagules when seed production of species fails for long periods.

Thompson and Grime (1979) described two general seed banks: transient and persistent. A transient bank is defined as one in which none of the seeds produced in a given year remain viable in the habitat for more than 1 year, whereas seeds in a persistent bank remain viable in the habitat for 1 or more years. Walck et al., (1996) have suggested that these two types of seed banks should be described in terms of germination season following maturation. Thus, a transient seed bank is composed of seeds that do not live until the second germination season following maturation, while a persistent seed bank is composed of seeds that live until the second (or some subsequent) germination season.

Artic and boreal/subalpine plant communities and coniferous and deciduous forests have relatively few persistent seeds, whereas pastures and arable soil have large numbers of them. Tropical savannas have more persistent seeds than most rainforests, and temperate grasslands or prairies have more than coniferous and deciduous forests (Baskin and Baskin 1998).

Not all species in a community are necessarily represented in the soil seed bank (Amiaud and Touzard 2004; Wills and Read 2007; Hopfensperger 2007; Chaideftou et al., 2009). Disturbances and fragmentation, for example, may influence species richness and abundance in the soil seed bank (Young et al., 1987; Grombone-Guaratini and Ribeiro-Rodrigues 2002). Soil seed bank densities also can be influenced by rates of seed predation and pathogen infection in the soil, by intrinsic rates of loss in viability following dispersal, and by variation in the timing and duration of fruit production (Dalling et al., 1997; Dalling 2004; Fornara and Dalling 2005).

The seed bank in the soil of an ecosystem can provide important information as to what species may have existed in the standing vegetation of the past and/or represent a pool of regenerative potential (Hopfensperger 2007). Numerous studies have examined above and below-ground species compositions in temperate (Thompson and Grime 1979) and tropical forests (Young et al., 1987; Drake 1998), grasslands (Peco et al 1998, Hui and Kepin 2006), arctic coastal marsh (Chang et al., 2001) but none have examined above and below-ground species composition of woody species in the tropical savannas of Brazil.

Seedling establishment

After a seed has germinated it gives rise to a seedling whose growth is largely dependent, at least for a time, on its own stored food reserves (Fenner 1985). As the growth of the shoot and root proceeds, dependence on internal resources is gradually reduced and external supplies of carbon and minerals begin to be exploited. Because of their small root systems, seedlings are extremely vulnerable to desiccation from minor soil drying events, so there is usually a selective pressure for rapid root extension (Lambers et al., 1998). In addition, too much or too little light (Matlaga and Horvitz 2009), soil water deficits (Hoffmann 1996), nutrient availability (Barloto et al., 2006; Breen and Richards 2008), leaf litter cover (Molofsky and Augspurger 1992), abundant grass biomass (Hagenah et al., 2009), predation, pathogens, burial, and competition highly constrain emergence and establishment of seedlings of woody species. Seedling establishment of neo-tropical savanna trees is particularly constrained by grass root competition, drought, and fire (Medina and Silva 1990). Therefore, successful establishment of savanna tree seedlings depends to a great extend on their capacity to reach moist soil layers beyond the grass root zone, and on the buildup of underground energy reserves, which allow re-growth of aerial biomass after fire or drought (Franco 2002).

Effects of Fire on plant reproduction

Fire affects plant growth, survival and dynamics of seeds and seedlings (Bond and Wilgen 1996). Thick bark, crown architecture, and resprouting from bark-protected buds or from subterranean organs all contribute to fire survival. Species with the most effective vegetative fire-survival traits have populations least influenced by fire. For species without such traits, fire-induced mortality is a key factor in regulating population growth and community composition (Bond and Wilgen 1996).

Fire has complex effects on plant reproduction. Many species are stimulated by fire, showing enhanced flowering, seed germination and seedling recruitment. Some have an obligate dependence on fire for reproduction and can be driven to extinction if fires are suppressed. For example, in fire-prone ecosystems such as Mediterranean shrublands, fire has been shown to improve conditions for seedling establishment (Tyler 1995). In these ecosystems, burning removes the inhibitory effects of established vegetation, such as accumulated litter, a closed canopy or allelopathic chemicals (Tyler 1995).

Fire often has been cited as a key factor in preventing seedling establishment (Bond 2008). For example, seedlings of many tropical savanna trees can only survive if fires are not too frequent and an interval of 2 to 3 years without burning seems to represent the minimum time necessary for the survival of some species (Hoffmann 1998). In a study of the effects of a biennial fire regime on the regeneration of *Blepharocalix salicifolius* in the cerrados of Brazil, Matos (1994) found twice the number of seedlings and juveniles in an area protected from fire for 18 years as in an area that was burned every two years. Species, however, vary greatly in seedling tolerance to burning (Bond 2008). In frequently burned savannas, for example, seedlings can acquire the ability to resprout within their first growing season (Hoffmann 1999, Midgley and Bond 2001) and clonal propagation from root suckers also is common, reducing dependence on seedlings (Lacey and Johnston 1990, Hoffmann 1999).

In several fire-prone ecosystems, heat from fires has been shown to stimulate seed germination, particularly in species with impermeable seed coats (Baskin and Baskin 1998; Overbeck et al., 2006). Fire-triggered germination is the result of either heat shock or chemical products of combustion, and species appear to utilize one or another of these germination cues as an indication of relatively competition-free sites for seedling establishment (Keeley and Fotheringha 1998). Variations in soil heating can affect recruitment because seeds have different tolerances to heating and different requirements for germination (Tyler 1995). Studies evaluating the effects of fire on seed germination of cerrado woody species are inconsistent; while some studies have shown positive effects of heat shocks of 70-100°C on seed germination of several woody species, in particular those with hard seed coats (Heringer 1971), other studies have shown negative or no significant effects of high temperatures on seed germination of some cerrado tree species (Rizzini 1976, Felippe and Silva 1984).

Objectives

The main objective of my Ph.D. research was to evaluate seed dynamics and seedling establishment of woody species in three major vegetation types in the savannas of central Brazil (cerrado). These vegetation types differ in tree canopy cover and tree height and are located along shallow topographic gradients of approximately 30 m in elevation and 1 km in length. I specifically addressed the following general questions:

1. Are cerrado vegetation types limited in seeds of woody species?
2. Do woody species form persistent soil seed banks along cerrado vegetation types?
3. Does seedling establishment of woody species differ among cerrado vegetation types?
4. Do richness and abundance of seedlings of woody and herbaceous species differ between fire-protected and burned vegetation types?

Each one of these four general questions is the focus of each of the four chapters of my Ph.D. dissertation. In this dissertation, I focused on early stages of the life cycle of woody species, which along with environmental factors may determine variations of tree density in the Brazilian savannas.

To find out if cerrado vegetation types are limited in seeds of woody species, in Chapter 2 of my dissertation, I studied spatial and temporal variations of the seed rain in three different vegetation types (closed, intermediate and open savannas). I assessed seed rain with 108 seed traps located in the field (36 per vegetation type). Using the seed rain data, I determined the degree of seed limitation of 23 woody species in each vegetation type. In the field, I also conducted experiments to determine the degree of seed predation and removal of 17 common cerrado woody species in each vegetation type. Finally, I determined seed viability loss of 12 of these common cerrado woody species in laboratory dry storage conditions.

Because soil seed banks may constitute an alternative source of propagules, in Chapter 3 I determined density and species composition of seeds found in the soil (soil seed bank) from each vegetation type during four consecutive soil collection periods including two dry and two wet seasons. I used the percentage of species re-occurring in the soil throughout all collection periods as indicative of degree of soil seed bank persistence. In Chapter 3, I also assessed kind of dormancy (or lack thereof), moisture content, and longevity of seeds of 14 common cerrado woody species which differ in their dispersal season. Finally, I examined similarity patterns in woody species composition among soil seed bank, seed rain, established seedlings, and standing vegetation in the vegetation types mentioned above.

In Chapter 4, I test the hypothesis that seedling establishment of woody species differs among cerrado vegetation types, even in the absence of major disturbances such as fire. To test this hypothesis, I evaluated density and survival of an initial seedling assemblage and of three consecutive cohorts of recruits of woody species within 216 permanent plots of $1m^2$ located in the three vegetation types. I quantified seedling limitation for 16 woody species as the proportion of the plots where seedlings of a particular species were not found. I also quantified light level, light quality, litter cover, soil moisture, and soil nutrient concentrations in each savanna type and performed manipulative experiments in a greenhouse to evaluate the effects of light level and litter cover on seedling emergence of nine common cerrado woody species.

Because fire is a major disturbance factor in tropical savannas, in Chapter 5 I evaluated the effect of fire on recruitment of seedlings and vegetative reproduction of woody and herbaceous cerrado species. In the field, I quantified richness and density of seedlings and vegetative sprouts (root suckers) in fire-protected savannas and burned savannas. Burned savannas comprised accidentally burned savannas differing in tree canopy cover and a frequently burned savanna with intermediate tree canopy cover. Because many plant species found in fire-prone ecosystems have seeds which require heating or other means of seed coat scarification for germination, in this chapter I also evaluated the effects of pulses of heat (heat-shocks) on seed germination of five common cerrado tree species under experimentally controlled conditions.

Chapter 2

Seed rain, seed predation and removal as determinants of seed limitation in Neotropical savanna trees

Spatial patterns of seed dispersal are crucial for determining the structure and dynamics of plant populations (Nathan and Muller-Landau, 2000). Seed dispersal is an important source of new individuals and determines potential sites of plant recruitment (Harper 1977). Experimental studies and theoretical models have been used to study the impact of seed dispersal on patterns of species distribution and biodiversity at local and landscape levels (Clark et al., 1998; Nathan and Muller-Landau 2000; Muller-Landau et al., 2002; Foster and Tilman 2003; Poulsen et al., 2007). The results of many of these studies suggest that some communities are seed-limited, meaning that micro-sites where seeds could arrive and germinate remain vacant (Clark et al., 1998; Hubbell et al., 1999). Seed-limited populations have fewer individuals than possible because seeds fail to arrive in saturating densities at potential recruitment sites (Muller-Landau et al., 2002). Many seed-sowing experiments show evidence of seed limitation in approximately 50% of the plant species studied (Turnbull et al., 2000) with early successional habitats and early successional small seeded-species exhibiting the highest seed limitation. However, some species with large seeds have been found to be more seed limited than small-seeded species (Moles and Westoby 2002). In a review of 43 studies, Clark et al., 2007 found that the addition of seeds to a site generally resulted in more established seedlings than in sites where no seeds were added.

Seed predation may reduce recruitment in populations that are limited by the availability of seeds rather than by microsites (Denham 2008). Seed predators are likely to have substantial effects on recruitment by reducing the number of seeds available for germination (Fenner and Thompson 2005; Campbell and Clarke 2006). The number of established seedlings increases when seeds are protected from predators (Terborgh and Wright 1994; Asquith et al., 1997; Perez et al., 2006). Seed predation can differ greatly among species, locations, and years, but it is often high. For example, rodents eat 99.7% of dispersed *Ocotea endreiana* seeds in montane Costa Rican forests (Wenny 2000). Faria (2004) found high predation and removal rates of *Qualea grandiflora* and *Kielmeyera coriacea* seeds in Brazilian savannas with 95% and 91% of *K. coriacea* seeds removed in open savannas and closed savannas, respectively, 60 days after dispersal. About 100% and 98% of *Q. grandiflora* seeds were removed by insects in open and closed savannas respectively. Secondary seed dispersal can play an important role in plant recruitment, because some seeds are transported intact by ants, dung beetles, scatter-hoarding animals, or abiotic processes to new microsites where germination is possible (Vander Wall et al., 2005). Rapid loss of seed viability that results in a decrease of the available seed pool also constrains recruitment of plant populations. The rate of seed viability loss over time is influenced by the identity of the species, moisture content of the seeds, temperature and period of storage (Fenner and Thompson 2005). Together post-dispersal processes of seed predation, removal, and seed viability loss can critically affect plant reproductive success and ultimately influence species distribution patterns.

The savannas of central Brazil (cerrado) cover approximately two million km^2, nearly 23% of the country. They are characterized by strong seasonality of precipitation and by well drained, deep, nutrient-poor soils (Furley 1999). More than 150,000 species of plants and animals are found in the cerrado making it a highly diverse ecosystem with a unique fauna and the largest plant diversity of all savanna floras in the world (Oliveira-Filho and Ratter 2002). Cerrado vegetation exhibits consistent changes in leaf area index, tree density and tree size along shallow topographic gradients of several meters in elevation (~30m) and a few kilometers in length. Vegetation structure varies from closed savannas with a relatively high density of tall trees in the upper portions of the gradient (cerrado denso) to open savannas with relatively few small trees in the lower portions of the topographic gradient (campo sujo). Other savanna types such as cerrado sensu stricto occupy intermediate topographic positions. Herbaceous plant abundance follows the opposite trend, with high abundance in open savannas and low abundance in closed savannas (Eiten 2001; Hoffmann et al., 2005).

Although reproductive phenology and seed dispersal syndromes have been described for cerrado communities (Mantovani and Martins 1988; Batalha et al., 1997; Oliveira and Gibbs 2002; Batalha and Martins 2004; Gottsberger and Silberbauer-Gottsberger 2006), few studies have evaluated seasonal variation of seed rain (e.g. Souza 1993; Marimon and Felfilli 2006), and none have estimated the degree of seed limitation among cerrado vegetation types. In this study I tested the following hypotheses: (1) variation in the number of species in the seed rain parallel the seasonal variation in precipitation, (2) number of diaspores in the seed rain is higher in closed than in open savannas, (3) the relative abundance of seeds with different dispersal mechanisms (i.e., wind, animal, gravity) in the seed rain reflect the dispersal syndromes of the local vegetation (4) seed limitation is higher in open savannas than in closed savannas, and (5) seed predation and removal from the ground, and rapid loss of seed viability constrain the total number of propagules available for germination.

Methods

Study site

This study was conducted from June 2005 until November 2007 at the Instituto Brasileiro de Geografia e Estatística (IBGE) reserve, a field station located 35 Km south of Brasilia, Brazil (15˚ 56' S, 47˚ 63' W, altitude 1100 m). Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August are often rainless (Figure 2.1). Relative humidity during the rainy season is about 80% and drops to 55% during the dry season when daily minimum relative humidity may reach values as low as 10% at midday. Mean monthly temperatures ranged from 19 to 23˚C. The soils are very deep and well-drained oxisols. The IBGE reserve contains all major vegetation types from very open to closed savannas. In June 2005, I established three transects (of approximately 1000 m) in the reserve, and each spanned the three major vegetation types. Transects were placed along continuous topographic gradients with different elevations (ranging from 1117 to 1153 m a.s.l.), in vegetation types that had been protected from fire for at least 30 years. I established nine plots of 20 x 20 m along each transect (three per vegetation type), for a total of 27 permanent plots. I determined preliminary species-area curves for cerrado denso and campo sujo areas, and the results indicated that about 85% of the maximum richness was attained at a plot size of 1024 m^2. Species composition and abundance in each plot was assessed by identifying and counting all individuals taller than 1 m.

Cerrado denso is a semi-closed woodland (40%-60% tree crown cover) with a moderately tall (usually 7 m) tree canopy. Because of the shade created by the canopy trees, the herbaceous and semi-woody ground vegetation has a lower cover than in relatively open cerrado vegetation. Cerrado sensu stricto is a savanna dominated by trees and shrubs (canopy generally less than 7 m tall) with 10 to less than 40% tree crown cover. Campo sujo has few short trees and scattered shrubs over a near continuous grass layer (the woody layer usually covers less than 10% of the ground).

Seed rain

To estimate seed arrival from reproductive trees and shrubs at the study sites, four seed traps were located at the corners of each of the 20 x 20 m plots for a total of 108 traps. Seed traps consisted of a plastic funnel 20 cm in diameter with a collection area of 0.0314 m^2 that was inserted into a PVC tube (5 cm diameter) that held the funnel neck (4.5 cm diameter) in place. Fruit and seed diameters of most cerrado woody species range from 0.3 to 50 cm and from 0.2 to 9 cm, respectively. Because few species in my field site exhibited fruit and seed diameters larger than 4.5 cm (3.3% and 6.02%, respectively), mesh bags in the seed traps were able to capture about 97% of fruits and 92% of seeds of cerrado woody species. The PVC tube was buried 20 cm into the ground. The height of the funnel opening was elevated 50 cm above the ground. A small nylon mesh bag (mesh size 0.1mm) was attached at the funnel base to collect the seeds or dispersal units. The PVC tube had several 5 mm diameter holes for drainage. All seeds, fruits and fruit parts (diaspores) were collected once a month over a 13 month period, from November 2005 until February 2007. The mesh bags were replaced when necessary. All material from each trap was examined under a dissecting scope. Whole, apparently intact diaspores were separated according to their morphologies as wind-dispersed or not wind-dispersed, then identified to species (or at least to family when it was not possible to identify the species), and dried to constant weight. Identification was based on published literature, by comparison with herbarium collections, and by comparison with reference specimens collected from reproductive plants.

Seed limitation

From the seed rain traps, fundamental seed limitation, source limitation, and dispersal limitation (Clark et al., 1998 and Muller-Landau et al., 2002) were calculated for woody species that had five or more seeds at least in one of the three vegetation types studied. Fundamental seed limitation (FSL) is the proportion of sites or traps not receiving seeds. FSL = 1- (a/n) where a is the number of traps receiving seeds of a particular species and n is the number of traps. FSL ranges from 0 to 1. A value of 1 indicates maximum limitation because seeds of a particular species are not captured in any trap. Because seed limitation can arise from limited seed numbers and/or limited distribution of available seeds among sites, seed limitation can be decomposed into source limitation and dispersal limitation (Clark et al., 1998). Source limitation (SL) is the failure of seeds to reach sites because of insufficient seed number assuming a uniform random distribution of seeds among traps. Uniform distribution is defined stochastically as a Poisson seed rain with equal expectation everywhere. The proportion of traps at which no seeds arrive under such a distribution is the Poisson probability of zero events given an expectation of s/n seeds. Thus, source limitation can be calculated as SL = $\exp^{(-s/n)}$ where s = number of seeds of a particular species and n is the number of traps. Under this stochastic definition, SL is non-zero even though there are more seeds than traps. The influence of restricted dispersal (Dispersal limitation) can be assessed by comparing the proportion of traps reached by seeds in reality with the proportion of traps that would be reached by seeds if dispersal were uniform. Thus, dispersal limitation (DL) which is seed limitation because of non-uniform distribution of seeds among sites can be calculated as DL = 1- ((a/n)/(1-source limitation)). DL ranges from 0 to 1. A value of 1 indicates maximum dispersal limitation.

Seed predation, removal, and viability

To quantify seed predation and seed removal I performed field experiments with diaspores collected from 17 woody species at the study site: *Brosimum gaudichaudii* (Moraceae), *Eriotheca pubescences* (Malvaceae), *Erythroxylum suberosum* (Erythroxylaceae), *Guapira graciliflora* (Nyctagynaceae), *Guapira noxia* (Nyctagynaceae), *Heteropterys byrsonimifolia* (Malpiguiaceae), *Miconia ferruginata* (Melastomataceae), *Mimosa clausenii* (Fabaceae), *Ouratea hexasperma* (Ochnaceae), *Palicourea rigida* (Rubiaceae), *Piptocarpha rotundifolia* (Astereaceae), *Protium ovatum* (Burseraceae), *Pterodon pubescens* (Fabaceae), *Rourea induta* (Connaraceae), *Schefflera macrocarpa* (Araliaceae), *Stryphnodendron adstringens* (Fabaceae) and *Styrax ferrugineus* (Styracaceae). Seeds or dispersal units (For simplicity I will refer as “seeds” to both true seeds and dispersal units) from at least three individuals were collected in the field and mixed to form a bulked sample. During the fruiting period of each species, seed removal was studied in all vegetation types of one transect.

Within each of the plots of this transect, seeds were scattered on four randomly selected sites within circular frames of 15 cm diameter. Each frame received 10 seeds of a single plant species. Frames were put on the soil after removing the litter at a distance of 10 cm from each other in a straight line. I recorded the number of missing seeds, as well as partially or total digested seeds every day for 30 or 45 days (depending on the species). I differentiated between these two fates because I cannot know if removed seeds suffered predation or were only secondarily dispersed. The experiments were performed during late wet season (February 2007), middle dry season (July 2007) and early wet season (November 2007).

Because seeds dispersed in dry and wet seasons may experience differential periods of drought in the field, I evaluated the rate of seed viability loss under laboratory dry-storage conditions. Seeds from twelve woody species were collected at the study site: *Acosmium dasycarpum* (Fabaceae), *Eriotheca pubescences* (Malvaceae), *Erythroxylum suberosum* (Erythroxylaceae), *Guapira graciliflora* (Nyctagynaceae), *Guapira noxia* (Nyctagynaceae), *Kielmeyera coriaceae* (Guttiferae), *Miconia ferruginata* (Melastomataceae), *Ouratea hexasperma* (Ochnaceae), *Palicourea rigida* (Rubiaceae), *Piptocarpha rotundifolia* (Astereaceae), *Solanum lycocarpum* (Solanaceae) and *Styrax ferrugineus* (Styracaceae). Fruits and seeds from at least three individuals were collected in the field and mixed to form a bulked sample. Every month, up to four or five months, seed viability percentage of each species was estimated by using a 1% Tetrazolium® solution (2, 3, 5-Triphenyltetrazolium chloride). Three samples of 50 seeds, previously stored in dry conditions (i.e., inside paper bags at room temperature) were used for each species every month. Seeds were soaked in the Tetrazolium solution in flasks totally wrapped with aluminum foil, which were placed in an oven at 40^{o}C. Embryo coloration patterns were evaluated under a dissecting scope every hour until embryos were completely stained. For the species of this study this time was between 12-24 hours.

Seeds were recorded as viable when embryos where homogeneously stained (i.e., both radicle and cotyledons).

Data analysis

Differences in fundamental seed limitation, source limitation and dispersal limitation among vegetation types were examined using one-way ANOVAs using the program JMP 7 (SAS Cary, NC). To fulfill the assumptions of normality, the data were transformed by calculating the arcsine of the square root of each original value. The data from the three transects of each vegetation type were pooled. Tukey's HSD post hoc test was used for multiple comparisons of means. Differences of these variables at the species level were examined using one-way ANOVAs for every species in each vegetation type. Both species means and grand means were examined. Differences in the final percentage of seed predation, seed removal, total seed loss and speed of removal (R^{50}) among vegetation types were examined with one-way ANOVAs. Tukey HSD post hoc tests were used for multiple comparisons of means. Differences of these variables at the species level also were examined using one-way ANOVAs for every species in each vegetation type. R^{50} was calculated as the number of days until 50% of the seeds had been lost (either preyed upon or removed); consequently, this index was only calculated for those species of which the total final loss was higher than 50%. Negative exponential and linear functions were fitted to the data of percentage seed viability loss as a function of time. The percentage of viability for each species after 12 months of storage was calculated based on the best fit equations.

Results

Seasonal variation in seed rain

During the study period, the dry season extended from April to September and the months of June, July and August received on average less than 10 mm of rain per month. At the beginning of the dry season the average monthly temperature decreased by 4˚C (Figure 2.1a).Consistent with this pattern of variation in precipitation and temperature, most diaspores were dispersed during the late dry season and early wet season in all three vegetation types (Figure 2.1b). Species diversity of diaspores, when taking into account both number of species and relative abundance, exhibited a pattern of seasonal variation similar to that of diaspore richness and abundance (data not shown). Seasonal patterns of richness and diversity were similar for the three vegetation types; however, the number of diaspores per m^2 was site-dependent with a higher number of diaspores in cerrado denso during the late dry season (July and August). The degree of seasonality in the richness of diaspores depended on the dispersal syndrome: animal-dispersed seeds exhibited less seasonal variations than wind-dispersed seeds (Figure 2.2). Seasonal variations in the abundance of anemochoric, zoochoric and autochoric diaspores followed the same pattern as species richness (results not shown).

Number of diaspores and dispersal syndromes in the seed rain

Throughout the period of study I trapped a total of 3,427 diaspores belonging to 41 species, 30 genera and 19 families (Table 2.1). Neither the mean number of species (diaspore richness) (One way anova; $F_{2,6} = 0.27$; $p = 0.77$), nor the mean number of diaspores per m^2 (One way anova: $F_{2,6} = 1.49$; $p = 0.77$) differed significantly among the three savanna types; however, the total number of diaspores was lower in campo sujo (693) than in cerrado denso (1,280) and cerrado sensu stricto (1,449). Species composition of diaspores differed among the savanna types. For example, the percentage of species belonging to Asteraceae was higher in campo sujo than in cerrado sensu stricto and cerrado denso. Conversely, the percentage of Melastomataceae species was higher in cerrado sensu stricto than in cerrado denso and campo sujo; and the percentage of Bignoniaceae species was higher in cerrado denso than cerrado sensu stricto and campo sujo (Table 2.1).

The relative abundance of woody species with animal-dispersed diaspores (zoochory) was higher in cerrado denso than in cerrado sensu stricto and campo sujo respectively (Table 2.2); conversely, the relative abundance of woody species with wind-dispersed diaspores (anemochory) was higher in campo sujo sites than in cerrado sensu stricto or cerrado denso respectively (Table 2.2).The relative abundance of the trapped seeds, characterized according to their dispersal modes, was consistent with the relative abundance of the dispersal syndromes of the species found in campo sujo and in cerrado sensu stricto. The relative abundance of the diaspores according to their dispersal syndromes in cerrado denso however, did not parallel those of the vegetation because of the high abundance of a wind-dispersed Bignoniaceae species that was over-represented in these sites during June and July 2006.

Seed limitation

Fundamental seed limitation was high across the three vegetation types for the 23 woody study species (Table 2.3). Significant differences among the three vegetation types ($p < 0.05$) were only found for five species: *Baccharis salzmanii* ($F = 7.79$, $p = 0.02$), *Bignoniaceae sp.* ($F = 15.72$; $p = 0.004$), *Byrsonima cf crassa* ($F = 7.00$, $p = 0.03$, *Miconia sp1* ($F = 12.30$; $p = 0.008$) and *Myrsine guianensis* ($F = 6.64$; $p = 0.03$). Of all these five species with significant differences in seed limitation among vegetation types, four of them had substantially higher seed limitation in campo sujo than in cerrado denso and cerrado sensu stricto. Dispersal seed limitation was also high for all woody species (Table 2.4). All species, except *Miconia sp1*, did not differ significantly ($p > 0.05$) across the three vegetation types. Dispersal limitation of *Miconia sp1* seeds was significantly higher in campo sujo than in the other two vegetation types ($F = 13.17$, $p = 0.006$) (Table 2.4). Mean fundamental seed limitation, source limitation and dispersal limitation across all species in table 4 was higher than 61% across vegetation types. Although no significant differences were found in mean fundamental, source, and dispersal seed limitations among vegetation types, these limitations were consistently higher in open than in closed savannas (Table 2.5).

Seed predation, removal, and viability

The percentage of seed predation and removal differed greatly among species. Seed predation ranged from 0 to 64.17% while seed removal ranged from 0 to 100% (Table 2.6). Mean seed predation in cerrado denso was 17.47±2.00 %; in cerrado sensu stricto was 15.78±1.67%; and in campo sujo was 18.62±1.78%, and these did not differ significantly among vegetation types (One way ANOVA; $F_{2,603} = 0.63$; $p = 0.53$). Seeds of most species were quickly removed from the ground (Figure 2.3; Table 2.7). For example, more than 60% of *Erythroxylum suberosum* seeds and *Piptocarpha rotundifolia* seeds were totally removed during the first fifteen days of the experiment (Figure 2.3). The final percentage of removal of *Piptocarpha rotundifolia* seeds was significantly higher in cerrado sensu stricto and campo sujo than in cerrado denso while the final percentage of removal of *Erythroxylum suberosum* seeds was significantly higher in cerrado sensu stricto than in campo sujo (Table 2.6). Mean seed removal in c. denso (49.70±2.90%) did not differ significantly from the seed removal in cerrado sensu stricto (55.00±2.88) nor in campo sujo (42.21±2.84) (One way ANOVA; $F_{2,603} = 5.06$; $p = 0.007$, Tukey HSD poshoc test). The speed of removal (R^{50} index) differed among species and vegetation types ranging from 1.66 ± 0.19 to 36.00 ± 9.00 (Table 2.7). The mean of R^{50} was marginally (p=0.047) significantly lower in cerrado sensu stricto (7.26 ± 0.72 days) than in campo sujo (8.18 ± 0.78 days) and cerrado denso (9.96 ±0.87 days). Significant differences ($p < 0.05$) in speed of removal across the three vegetation types were found for only five species: *E. pubescens, G.noxia, M.clausenii, P.rigida* and *S.macrocarpa* (Table 2.7). Mean final seed removal was significantly higher in cerrado sensu stricto (55.00%) than in campo sujo (42.21%). Seed loss because of both predation and removal was significantly higher in cerrado sensu stricto (70.78%) than in campo sujo (60.83%). Neither cerrado sensu stricto nor campo sujo, however, differ significantly from cerrado denso (67.17%) in their total seed loss (One way ANOVA; $F_{2,603} = 3.67$; $p = 0.026$; Tukey HSD poshoc test).

Initial viability percentage and rate of viability loss differed among the species (Table 2.8). Negative exponential and linear functions were fitted to the data for percentage viability loss as a function of time. The coefficient of determination (R^2) of the functions ranged from 0.75 to 0.99 and they were significant at $p \leq 0.02$. With these functional relationships I predicted the percentage of viability for each species after 12 months of storage. Out of the twelve species, *Acosmium dasycarpum*, *Palicourea rigida*, *Erythroxylum suberosum* and *Guapira graciliflora* were the only species that maintained their viability after 12 months of storage, but at levels lower than 15%.

Discussion

Seasonal variation in the seed rain

Strong seasonal variations in the seed rain in the three vegetation types were observed with most woody and herbaceous species being dispersed during the mid and late dry season (August and September) and early wet season (October and November) (Figure 2.1). These seasonal patterns of seed rain support our initial hypothesis and are consistent with fruit phenology of cerrado plant communities where most woody species fruit mainly during the late dry season and early wet season (Batalha and Mantovani 2000; Oliveira and Gibbs 2002; Gottsberger and Silberbauer- Gottsberger 2006; Munhoz and Felfili 2007). Cerrado species exhibit variations in flower and fruit production that are apparently determined by seasonal variations in precipitation and humidity (Lenza and Klink 2006). I found a larger proportion of wind-dispersed species and wind-dispersed diaspores than animal- dispersed species and animal dispersed-diaspores during the dry season. Other studies with herbaceous and sub-shrub species in campo sujo and cerrado (Gouveia and Felfili 1998), and woody species in cerrado sensu stricto (Lenza and Klink 2006) also found that although leaves and flowers appear at all times of the year, their production is intensified during the transition between the dry and wet seasons. They also have reported that fruit maturation of autochoric and anemochoric species occurred within the dry season; zoochoric species dispersed seeds mainly during the wet season. Because wind-dispersed propagules tend to be drier than animal dispersed ones, drought, wind, and leaf fall may facilitate seed releasing mechanisms such as dehydratation of fruit pericarps followed by seed dispersal (Batalha and Martins 2004). Animal dispersed species exhibited less seasonal variation than did wind-dispersed species, a pattern also found in other phenological and reproductive studies of cerrado woody species (Souza, 1993; Batalha and Martins 2004).

The more stable seed rain of animal dispersed species and diaspores throughout the year could result from an extended maturation period of their fleshy fruits compared to the relatively smaller and drier fruits of anemochoric species. It could also suggest that animal dispersed fruits remain available for long periods of time throughout the year, thus increasing their chances of being dispersed (Mantovani and Martins 1988; Batalha and Mantovani 2000; Gottsberger and Silberbauer- Gottsberger 2006). Animal activity tends to be higher during the rainy season than during the dry season. Temporal variation in seed dispersal would be of little consequence if there is a large persistent soil seed bank (seeds living longer than one year in the soil). In a parallel study, I found that more than 50% of the species found in the soil of each vegetation type form transient soil seed banks and only 20 % of the species form persistent banks (results not shown). Consequently, the seasonal patterns of seed dispersal should have substantial consequences for population dynamics of woody plants in the cerrado.

Number of diaspores in the seed rain and dispersal syndromes

About 35% of the woody species found in the vegetation also were found in the annual seed rain (41 species of the seed rain out of the 117 species within the three vegetation types). Despite the low percentage of woody species found in the seed rain, this percentage could represent an important source of seeds for potential recruitment. The total number of diaspores captured with the seed rain traps during the period of study was lower in open (campo sujo) than in closed savannas (cerrado denso) supporting our second hypothesis. The difference in the total number of seeds captured could be the result of differences in tree density and tree canopy cover among vegetation types.

In this study wind-dispersed seeds were found in seed traps in larger proportions than animal-dispersed seeds despite a larger percentage of zoochoric than anemochoric species in the local vegetation. In general, wind and gravity dispersed seeds are better represented in seed traps than animal dispersed seeds (Kollmann and Goetze 1998). Almost 70% of the species found in both cerrado denso and cerrado sensu stricto areas rely on animals for seed dispersal (Table 2.2). On the other hand, the predominance of anemochorous and autochorous dispersal modes over zoochory in campo sujo areas indicates that less than half of the species in this particular vegetation type depend on animals for dispersing their seeds. In general, gravity (autochory) dispersal was not well represented in the vegetation, and water dispersed seeds (hydrochory) as expected, were not found among the species studied.

The relative abundance of the seeds, according to the dispersal syndromes, reflected the relative abundance of local woody species characterized by their dispersal syndromes, particularly in campo sujo and in cerrado sensu stricto (Table 2.2). This consistency, suggests that the local vegetation is an important source of seeds. Empirical data collected with seed traps are usually limited to short-distance events because of the inherent difficulty of sampling long-distance seed dispersal (Portnoy and Wilson 1993; Stevenson and Vargas 2008). The relative abundance of the seeds according to their dispersal syndromes in cerrado denso, however, did not reflect the dispersal syndromes of the species found there. This disparity could be explained by the high abundance of seeds of an anemochoric species (Bignoniaceae) that was over-represented in these sites during June and July 2006 (dry season) (Table 2.1).

The low percentage of animal dispersed seeds found in cerrado denso could also be the result of a large proportion of endozoochoric species (dispersal inside the animal) rather than epizoochoric (dispersal outside the animal), which would impede seeds from being collected by the traps. Indeed, in a cerrado area of Sao Paulo State in Brazil, Gottsberger and Silberbauer- Gottsberger (2006) found that the relative abundance of endozoochoric species in cerrado denso areas was significantly higher than in cerrado sensu stricto areas. As the proportion of woody versus herbaceous species tends to increase in the closed savanna formations, so does the proportion of zoochoric versus anemochoric and autochoric species (Oliveira and Moreira 1992; Gottsberger and Silberbauer- Gottsberger 2006).Wind dispersal has been generally associated with open vegetation like campo sujo, whereas in closed vegetation a more even distribution among dispersal syndromes has been found (Munhoz and Felfili 2007).Taking out the anemochoric species (Bignoniaceae) from cerrado denso, the results support our third hypothesis.

Seed limitation

I found that most woody species in our study exhibit high (>40%) seed limitation (Tables 2.3 and 2.4). None of the woody species was able to saturate the forest floor with its seeds. As a result, high fundamental seed limitation (percentage of sites not receiving seeds), high source limitation (failure of seeds to reach a site because of low seed abundance,) and high dispersal limitation (failure of seeds to reach a site because of non-uniform distribution among sites) of woody species significantly constrain the potential recruitment of seedlings. High seed limitation might partially explain the lack of a continuous canopy of trees and a multi-strata structure characteristic of forest ecosystems. Our results agree with those found by Foster and Tilman (2003) for savanna plant communities limited by seed availability. Mean fundamental seed limitation, source limitation and dispersal limitation across species were consistently higher in open than in closed savannas (Table 2.5) supporting our hypothesis. The total number of diaspores captured with the seed rain traps during the period of study was also lower in open savannas (campo sujo) than in closed savannas (cerrado denso and cerrado sensu stricto) (Table 2.1). The overall higher seed limitation along with the lower number of total diaspores (woody and herbaceous) in the open savanna than in the closed savannas might contribute to maintain the relative low tree density on the low parts of the topographic gradient. Because seed limitation slows the rates of change in plant abundance, the failure of seeds to arrive at all suitable sites might help to maintain the balance between grasses and a discontinuous tree cover because species cannot increase as quickly if they do not reach all suitable sites, nor as a result, do their competitors decrease as quickly (Muller-Landau et al., 2002). Seed limitation also may be a factor in the resilience of cerrado vegetation. The resilience of cerrado vegetation has been reported by Felfili et al., (2000) over a nine year period of continuous floristic surveys in a sample of 19 (20m x 50m) plots, the authors found little change (in the range of 5%) in species composition, spatial distribution, and community structure in a cerrado sensu stricto area. Future studies should be conducted to examine the relative importance of seed limitation at different distances from parents by comparing the seedling yield with the observed seed-dispersion pattern.

Seed predation, removal, and viability

Even if seed rain is underestimated by seed trap collection, post-dispersal processes such as predation and removal of seeds from the ground could significantly further reduce the number of seeds available for germination confirming our hypothesis. Most seeds were rapidly predated and removed from the ground in a field experiment done with seeds of 17 woody species, which should substantially lower recruitment rates. Seed predation also has been found to contribute to seed-limited recruitment in temperate woodlands in Australia (Campbell and Clarke 2006) and in Southeastern Australia fire-prone ecosystems (Denham 2008) by reducing the number of seeds stored in the soil available for germination.

Although seed removal cannot be considered a proxy for seed predation, because some seeds could be transported intact by ants, scatter-hoarding animals, or abiotic agents to new microsites where germination is possible (Vander et al., 2005), the high and fast seed removal suggests that secondary dispersal might play a major role in seed dynamics of cerrado woody species. Conspicuous interactions between fungus-growing ants

(Attini) with fruits and seeds of several cerrado species (personal field observation), suggest that ants may play a relevant role in seed biology in the cerrado. Potential ant-derived benefits to diaspores of non-myermecochorous plants in the cerrado would include secondary seed dispersal and/or increased germination success by ant-handled seeds (Leal and Oliveira 1998; Christianini et al., 2007). The significance of enhanced seed removal rates and distance dispersal for seedling establishment is unclear because the benefits to plants of having their seeds dispersed by ants in the cerrado are poorly known. Secondary dispersal by ants may reduce predation by rodents, reduce parent-offspring conflict and sibling competition, and may facilitate the location of favorable seed microsites (Parr et al., 2007).

Even if dispersed seeds avoid being eaten or attacked by pathogens in a safe microsite, rapid viability loss could further reduce the number of propagules available for plant recruitment. For the seeds of the twelve woody species included in this study, only 33% of them maintained viability for a period longer than 12 months, but at very low percentages (less than 15%). Interestingly, none of these long-lived seeds were found in the soil seed bank (Salazar, unpublished information) and this could be the result of high predation (Table 2.6). This rapid seed viability loss also could prevent the formation of persistent soil seed banks which in turn would reinforce seed limitation. Studies performed across different sites and species (Turnbull et al., 2000; Zobel et al., 2000; Moles and Westboy 2002) have shown that large-seeded species, species in disturbed microsites, and species with relatively short-lived seed banks exhibit high seed limitation. Indeed, short-lived seed banks should further increase the magnitude of seed limitation because viable seeds do not accumulate in the soil over time and cannot recruit into the population even during a productive seed year for that species (Deveny and Fox 2006; Clark et al., 2007).

Overall, the results of this study showed that in all three vegetation types: (1) temporal variation in the number of species in the seed rain, in particular species that are wind-dispersed, paralleled the seasonal variation in precipitation; (2) total number of trapped seeds during the study period was higher in closed than in open savannas; (3) dispersal modes of seeds in intermediate and open savannas were highly consistent with dispersal syndromes of the local vegetation, with more animal dispersed seeds and species in intermediate savannas and more wind-dispersed seeds and species in open savannas; 4) open savannas were particularly highly limited in seeds of woody species because of low seed numbers and seeds poorly dispersed among sites; 5) post-dispersal factors of seed predation, high and fast seed removal, and rapid loss of viability further increased seed limitation in all three vegetation types. In conclusion, potential rates of recruitment of cerrado woody species across vegetation types seem to be limited simultaneously by low seed supply and post-dispersal events of predation, removal, and rapid loss of viability. This study also showed that early stages of the plant life cycle of woody species should be considered among determinants of savanna structure and its variations along topographic gradients.

Table 2.1. Total number of diaspores of each of the species (herbaceous and woody) found in seed traps located in three vegetation types: cerrado denso, cerrado sensu stricto, and campo sujo during 13 months of observations. Species are grouped according to their dispersal syndromes: zoochory, anemochory and autochory.

Syndrome	**Species**	**Family**	**cerrado denso**	**cerrado sensu stricto**	**campo sujo**
Zoochory	*Byrsonima cf crassa*	Malpighiaceae	5	1	0
	Byrsonima sp.	Malpighiaceae	63	5	3
	Cordiera sp.	Rubiaceae	16	0	0
	Davilla elliptica	Dilleniaceae	28	109	25
	Guapira graciliflora	Nyctagenaceae	0	5	0
	Miconia albicans	Melastomataceae	0	42	0
	Miconia fallax	Melastomataceae	48	119	46
	Miconia ferruginata	Melastomataceae	0	3	0
	Miconia sp1	Melastomataceae	47	107	19
	Miconia sp2	Melastomataceae	18	22	8
	Miconia sp3	Melastomataceae	0	13	0
	Myrsine guianensis	Myrsinaceae	35	549	4
	Ouratea hexasperma	Ochnaceae	1	15	10
	Rourea induta	Connaraceae	17	17	10
	Styrax ferrugineus	Styracaceae	5	15	21
	Stryphnodendron adstringens	Fabaceae	0	4	11
	SUBTOTAL		**283**	**1026**	**166**
Anemochory	*A.dasycarpum*	Leguminosae	0	0	3
	A.macrocarpa	Apocynaceae	9	23	0
	Asteraceae sp.1	Asteraceae	0	0	22
	Asteraceae sp.2	Asteraceae	1	121	0
	Asteraceae sp.3	Asteraceae	16	2	8
	Baccharis salzmannii	Asteraceae	30	78	181
	Dalbergia miscolobium	Fabaceae	4	20	0
	Eremanthus glomerulatus	Asteraceae	78	68	90
	Eremanthus goyazensis	Asteraceae	10	12	5
	Eremanthus spp.	Asteraceae	6	4	7
	Eriotheca pubescens	Bombacaceae	12	2	0
	Heteropterys sp 1	Malpighiaceae	22	3	0
	Heteropterys sp 2	Malpighiaceae	6	9	0
	Jacaranda ulei	Bignoniaceae	0	0	6
	Kielmeyera coriaceae	Clusiaceae	0	6	15
	Bignoniaceae *sp.*	Bignoniaceae	434	0	0
	Pera glabrata	Euphorbiaceae	180	0	8
	Piptocarpha rotundifolia	Asteraceae	4	40	8
	Qualea sp.	Vochysiaceae	0	0	4
	Rhynchospora exaltata	Cyperaceae	135	0	0
	Thrasya spp.	Poaceae	13	0	1
	Vernonia fruticulosa	Asteraceae	26	25	48
	Vochysiaceae sp.	Vochysiaceae	0	1	2
	SUBTOTAL		**986**	**414**	**416**
Autochory	*Echinolaena inflexa*	Poaceae	11	9	97

Mimosa claussenii	Fabaceae	0	0	14
SUBTOTAL		11	9	111
TOTAL		**1280**	**1449**	**693**

Table 2.2 Abundance (%) of woody species (Plants) classified according to their dispersal syndrome (zoochory, anemochory, autochory) across three vegetation types: cerrado denso, cerrado sensu stricto and campo sujo, and relative abundance (%) of diaspores (seeds) of woody species collected from seed traps located in each of the above sites that were animal, wind, and gravity dispersed.

Dispersal syndrome		**cerrado denso**	**cerrado sensu stricto**	**campo sujo**
Zoochory	Plants	71.06	67.69	44.07
	Seeds	22.30	77.85	27.85
Anemochory	Plants	25.11	28.35	49.58
	Seeds	66.04	22.15	64.60
Autochory	Plants	0.50	0.42	3.66
	Seeds	0.00	0.00	2.35

Table 2.3. Fundamental seed limitation ± standard deviation (n=3) Proportion of seed traps not receiving seeds for 23 woody species in cerrado denso, cerrado sensu stricto and campo sujo. Diaspores from species of all vegetation types were included in this table. A value of 1 indicates maximum limitation because seeds of that particular species were not found in seed traps during the study. Bold rows indicate species that differ significantly according to Tukey HSD post hoc test. For those species, means with the same letter do not differ significantly among vegetation types.

Species	cerrado denso	cerrado sensu stricto	campo sujo
Baccharis salzmannii	**0.83±0.08 a**	**0.89±0.13a**	**0.50±0.17 b**
Bignoniaceae sp.	**0.89±0.05b**	**1.00±0.00a**	**1.00±0.00a**
Byrsonima cf crassa	**0.92±0.00b**	**0.97±0.05ab**	**1.00±0.00a**
Byrsonima sp.	0.78±0.13	0.94±0.05	0.94±0.05
Dalbergia miscolobium	0.94±0.05	0.97±0.05	1.00±0.00
Davilla elliptica	0.70±0.52	0.86±0.05	0.94±0.05
E.glomerulatus	0.67±0.30	0.56±0.26	0.47±0.34
Eremanthus sp.	0.92±0.00	0.92±0.14	0.92±0.08
Heteropterys sp 1	0.89±0.13	0.95±0.05	0.94±0.05
Heteropterys sp 2	0.89±0.19	0.94±0.05	1.00±0.00
Kielmeyera coriaceae	1.00±0.00	0.97±0.05	0.86±0.24
Miconia albicans	1.00±0.00	0.97±0.05	1.00±0.00
Miconia fallax	0.78±0.17	0.53±0.19	0.81±0.17
Miconia sp 1	**0.78±0.13a**	**0.42±0.17b**	**0.94±0.10a**
Miconia sp 2	0.72±0.05	0.83±0.17	0.94±0.10
Myrsine guianensis	**0.75±0.29ab**	**0.33±0.17b**	**0.89±0.05a**
Ouratea hexasperma	0.97±0.05	0.97±0.14	0.94±0.10
Pera glabrata	0.89±0.13	1.00±0.00	0.91±0.08
Pitptocarpha rotundifolia	0.92±0.08	0.86±0.13	0.88±0.09
Rourea induta	0.94±0.05	0.89±0.13	0.89±0.19
Stryphnodendron adstringens	1.00±0.00	0.97±0.05	0.94±0.10
Styrax ferrugineous	0.87±0.10	0.92±0.00	0.92±0.00
Vernonia fruticulosa	0.92±0.08	0.83±.014	0.72±0.21

Table 2.4. Dispersal limitation ± standard deviation (n=3) (Proportion of seed traps that would have been reached under random dispersal, but were not reached) for all woody species in the three vegetation types: cerrado denso, cerrado sensu stricto and campo sujo. Diaspores from species of all vegetation types were included in this table. A value of 1 indicates maximum limitation because seeds of that particular species were not found in seed traps during the study. Bold rows indicate species that differ significantly according to Tukey HSD post hoc test. For those species, means with the same letter do not differ significantly among vegetation types.

Species	**cerrado denso**	**cerrado sensu stricto**	**campo sujo**
Baccharis salzmannii	0.47±0.41	0.88±0.15	0.45±0.10
Bignoniaceae sp.	0.57±0.53	1.00±0.00	1.00±0.00
Byrsonima cf crassa	0.30±0.26	0.67±0.58	1.00±0.00
Byrsonima sp.	0.43±0.39	0.57±0.51	0.49±0.50
Dalbergia miscolobium	0.54±0.51	0.97±0.06	1.00±0.00
Davilla elliptica	0.88±0.21	0.53±0.50	0.62±0.58
E.glomerulatus	0.57±0.26	0.44±0.14	0.29±0.28
Eremanthus sp.	0.36±0.32	0.71±0.51	0.66±0.30
Heteropterys sp 1	0.76±0.42	0.49±0.50	0.80±0.19
Heteropterys sp 2	0.72±0.50	0.82±0.16	1.00±0.00
Kielmeyera coriaceae	1.00±0.00	0.93±0.12	0.80±0.34
Miconia albicans	1.00±0.00	0.97±0.05	1.00±0.00
Miconia fallax	0.64±0.17	0.50±0.15	0.77±0.20
Miconia sp 1	**0.67±0.07ab**	**0.34±0.20b**	**0.93±0.12a**
Miconia sp 2	0.25±0.20	0.61±0.38	0.89±0.20
Myrsine guianensis	0.36±0.35	0.33±0.17	0.03±005
Ouratea hexasperma	0.67±0.57	0.88±0.20	0.75±0.43
Pera glabrata	0.58±0.52	1.00±0.0	0.67±0.38
Pitptocarpha rotundifolia	0.75±0.44	0.78±0.20	0.61±0.34
Rourea induta	0.89±0.10	0.55±0.34	0.80±0.34
Stryphnodendron adstringens	1.00±0.00	0.90±0.17	0.91±0.16
Styrax ferrugineous	0.38±0.54	0.44±0.43	0.30±0.52
Vernonia fruticulosa	0.94±0.11	0.61±0.50	0.48±0.21

Table 2.5. Mean values of fundamental seed limitation, source limitation and dispersal limitation for vegetation type: cerrado denso, cerrado sensu stricto and campo sujo using all species listed in Table 3. Values are means ± SE. *F* values and *p* values from separate one-way ANOVA tests are shown in the last column.

	cerrado denso	**cerrado sensu stricto**	**campo sujo**	***F*($_{2, 204}$); *p***
Seed limitation	0.88 ± 0.02	0.85 ± 0.03	0.89 ± 0.02	0.95; $p = 0.38$
Source limitation	0.69 ± 0.04	0.65 ± 0.05	0.76 ± 0.04	1.71; $p = 0.18$
Dispersal limitation	0.61 ± 0.04	0.70 ± 0.04	0.72 ± 0.04	2.21; $p = 0.11$

Table 2.6. Percent (%) of seed predation and seed removal for 17 woody species in cerrado denso, cerrado sensu stricto, and campo sujo. Values are means ± SE (n=12). A total of 360 seeds were used for each species. Bold numbers indicate species that differ significantly in predation or removal among vegetation types according to Tukey HSD post hoc test. For those species, means with the same letter do not differ significantly among vegetation types.

Species	**Predation**			**Removal**		
	cerrado denso	cerrado sensu stricto	campo sujo	cerrado denso	cerrado sensu stricto	campo sujo
B.gaudichauidii	5.00 ± 1.95	7.50±2.18	17.50±6.98	**1.67±1.12 b**	**29.17±9.24 a**	**1.67±1.12 b**
E. pubescens	0.83±0.83	0±0	1.67±1.12	95.83±3.36	95.00±5.00	90.00±4.08
E.suberosum	13.33±8.34	20.00±9.92	25.00±10.11	**69.17±11.51ab**	**75.83±11.11a**	**40.83±11.45b**
G.graciliflora	0.00	0.00	0.00	90.00±6.10	97.50±1.30	99.17±0.83
G.noxia	**39.17±7.01ab**	**44.17±5.43a**	**21.67±6.0b**	**55.00±5.97ab**	**47.50±5.10b**	**70.83±6.80a**
H.byrsonimifolia	24.17±8.30	30.83±8.21	27.50±5.80	43.33±10.40	58.33±8.60	50.00±9.21
M.ferruginata	8.33±2.97	13.33±5.12	13.08±5.11	15.00±4.35	25.83±6.33	25.00±4.75
M.claussenii	**55.00±12.73a**	**25.83±6.57ab**	**21.67±5.23b**	12.50±7.40	12.50±8.97	0.83±0.83
O.hexasperma	0.00	0.00	0.00	**97.50±1.95a**	**100±0.00a**	**88.33±4.41b**
P.rigida	27.50±5.62	22.50±4.94	25.00±5.15	**68.33±4.91ab**	**76.67±4.66a**	**58.33±3.66b**
P.rotundifolia	0.00	0.00	0.00	**59.17±5.96b**	**87.50±3.29a**	**91.67±4.74a**
P.ovatum	11.67±8.24	23.33±10.40	45.83±11.31	**62.50±12.56a**	**60.83±13.56 a**	**14.17±7.93 b**
P.pubescens	0.00	0.00	0.00	0.00	0.00	0.00
R.induta	9.17±4.68	8.33±4.41	18.33±7.16	56.67±13.00	75.00±8.83	60.00±10.59
S.macrocarpa	**24.17±10.11 b**	**39.17±9.33 b**	**64.17±5.70 a**	**72.50±10.45a**	**47.50±12.32a**	**6.67±1.88b**
S.adstringens	35.00±8.66	20.83±7.01	16.67±4.82	0.83±0.83	3.33±1.88	0.83±0.83
S.ferrugineus	35.00±10.26	12.50±4.94	22.50±5.38	28.33±9.11	42.50±11.55	19.17±6.79

Table 2.7. Speed of loss because of seed predation and seed removal calculated as the number of days at which 50% of the seeds are predated or removed (R^{50}) for 13 woody species that exhibited a final loss higher than 50%. Experiments were done in cerrado denso, cerrado sensu stricto, and campo sujo. Values are means ± SE (n=12). A total of 360 seeds were used for each species. Bold rows indicate species that differ significantly according to Tukey HSD pos hoc test. For those species, means with the same letter do not differ significantly among vegetation types.

Species	**R^{50} (days)**		
	cerrado denso	cerrado sensu stricto	campo sujo
E. pubescens	**6.58±0.72 a**	**3.91±1.51 b**	**5.41±2.84 ab**
E.suberosum	14.90±4.07	9.33±1.57	12.63±1.87
G.graciliflora	2.64±0.58	2.42±0.50	3.58±0.51
G.noxia	**1.75±0.22 b**	**1.66±0.19 b**	**5.00±0.74 a**
H.byrsonimifolia	7.30±1.90	5.83±1.26	5.25±0.91
M.claussenii	**18.33±2.46 b**	**33.00±3.80a**	**36.00±9.00 a**
O.hexasperma	2.91±0.34	2.66±0.33	3.00±0.56
P.rigida	**6.17±1.11b**	**4.57±0.70 b**	**9.55±1.01 a**
P.rotundifolia	5.22±0.70	5.33±0.94	7.17±0.63
P.ovatum	24.89±5.29	13.00±2.96	26.00±6.96
R.induta	8.64±2.44	4.42±1.23	5.80±1.62
S.macrocarpa	**16.67±3.46 a**	**6.82±0.95 b**	**3.21±1.12 b**
S.ferrugineus	15.50±2.53	16.56±4.73	17.57±5.88

Table 2.8. Adjusted functions for seed viability loss as a function of time for twelve species. Linear and exponential functions were adjusted from the means of initial cohort of seeds (three repetitions of 50 seeds each).

Species	**Function**	**R^2**	**F**	**df**	***p***
Acosmium dasycarpum	y =87.27 -6.50*x	0.99	197.18	(1, 3)	0.005
Eriotheca pubescens	y = 90.06-15.50*x	0.996	493.12	(1,2)	0.002
Erythroxylum suberosum	y = 79.47*exp(-0.1652*x)	0.849	16.90	(1,3)	0.02
Guapira graciliflora	y = 57.53 -3.52*x	0.967	87.38	(1,4)	0.003
Guapira noxia	y= 78.44*exp(-1.13*x)	0.968	93.19	(1,3)	0.002
Kielmeyera coriaceae	y =104.82 -20.23*x	0.93	40.08	(1,4)	0.008
Miconia ferruginata	y = 9.67-1.10*x	0.956	86.84	(1,4)	0.001
Ouratea hexasperma	y =62.5300 -6.8920*x	0.778	10.51	(1,4)	0.048
Palicourea rigida	y = 56.86-3.81*x	0.875	27.91	(1,4)	0.006
Pipthocarpa rotundifolia	y= 9.78*exp(-0.48*x)	0.747	11.85	(1,4)	0.02
Solanum lycocarpum	y = 77.80-10.07*x	0.981	159.03	(1,3)	0.001
Styrax ferrugineus	y= 37.81*exp(-0.48*x)	0.974	50.49	(1,3)	0.005

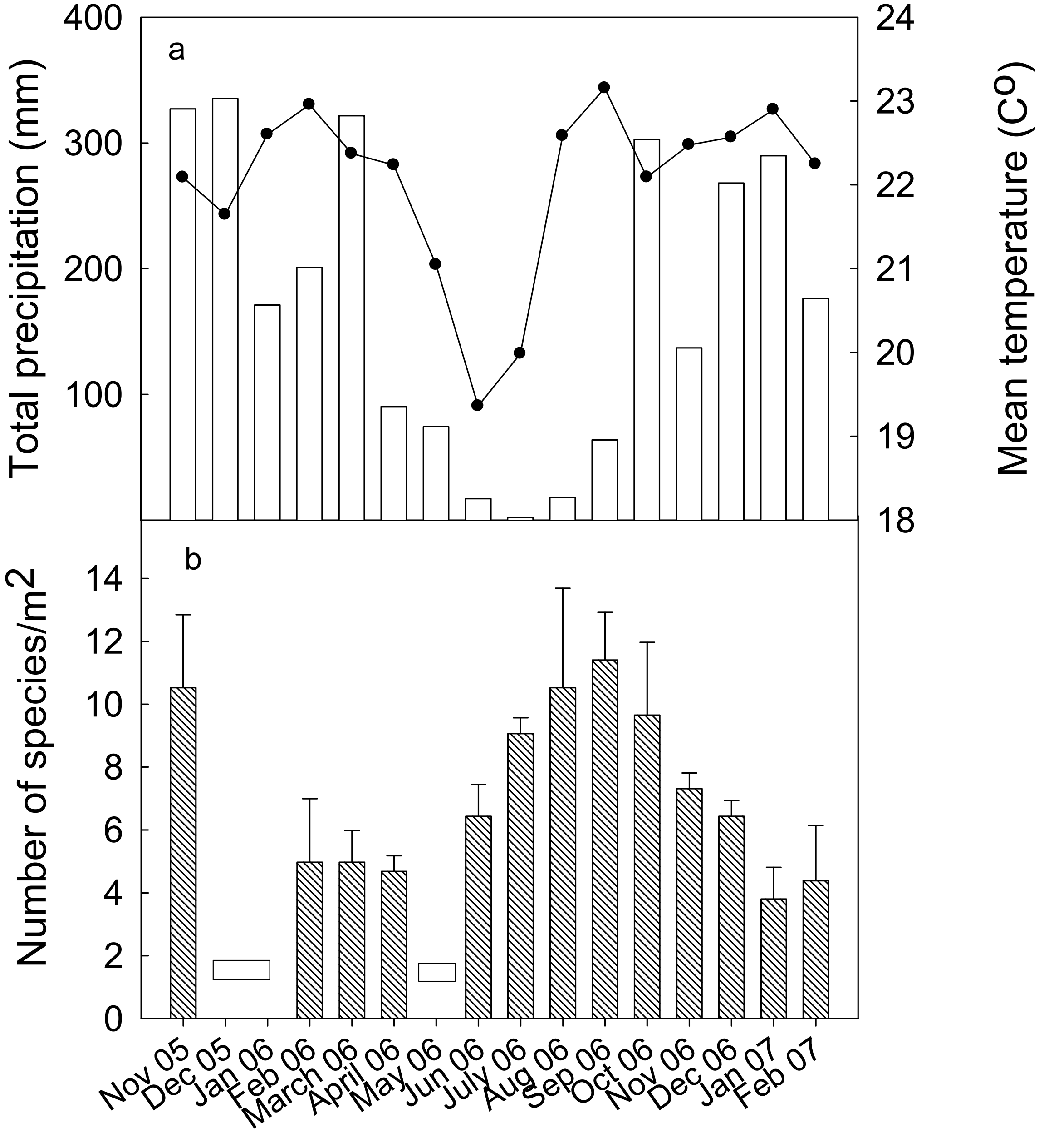


Figure 2.1 a) Total monthly precipitation (bars) and mean monthly temperature (line and solid symbols) from November 2005 until February 2007 obtained from the IBGE meteorological station located near the study sites. b) Mean number of species per m^2 of the three vegetation types from November 2005 until February 2007. Bars are means of the total number of diaspores of herbaceous and woody species trapped in cerrado denso, cerrado sensu stricto and campo sujo. Empty boxes indicate months without measurements.

Figure 2.2. Number of anemochoric and zoochoric species per m^2 from November 2005 until February 2007. Bars are total number of diaspores of herbaceous and woody species trapped in cerrado denso (c.denso), cerrado sensu stricto (c.s.s), and campo sujo (c.sujo). Bars represent total number species per m^2 found in all the seed traps in the three vegetation types. Empty boxes indicate months without measurements.

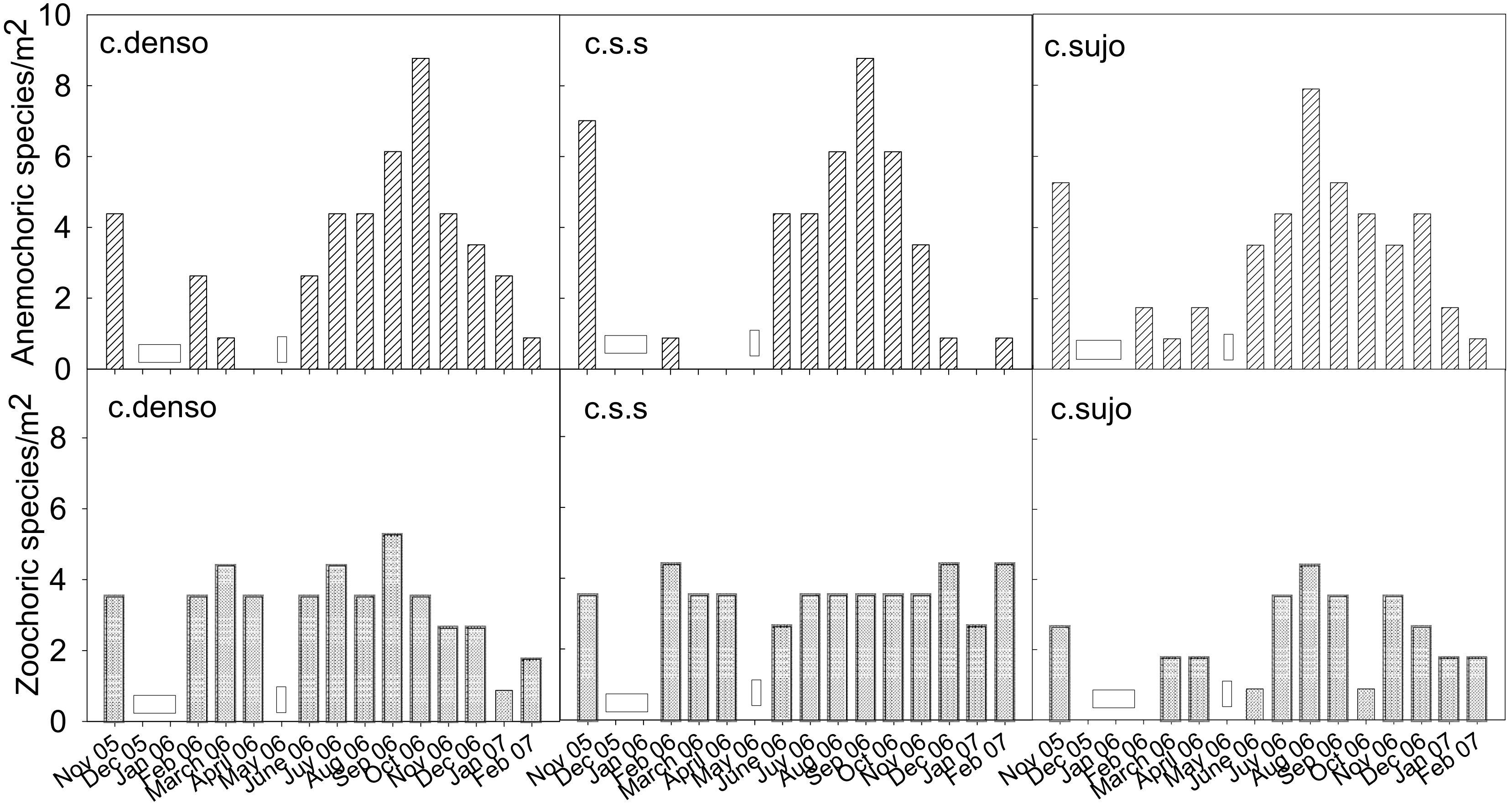
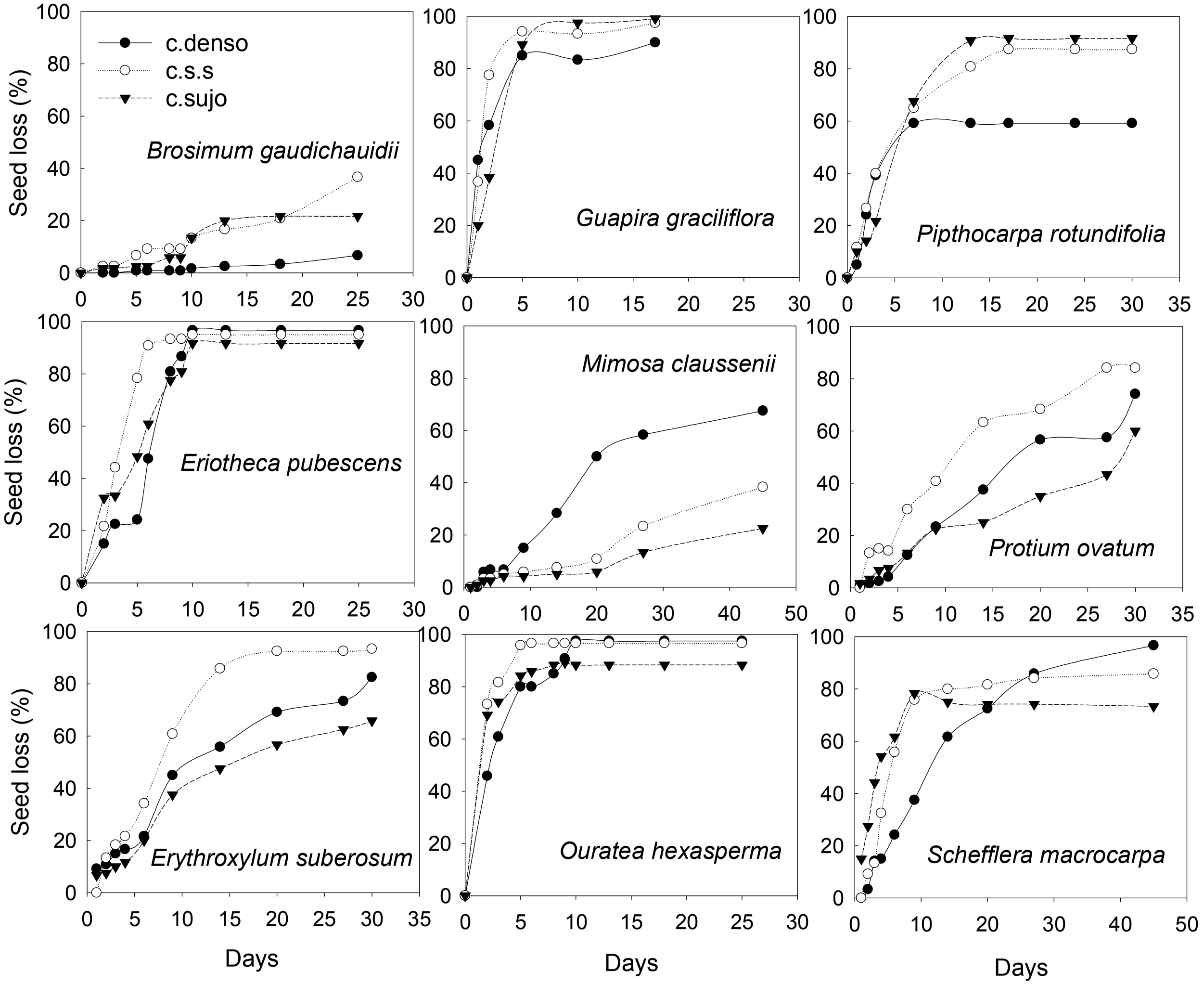


Figure 2.3. Seed loss percent (%) (Predation plus removal) of species that differ significantly (except *Eriotheca*) among vegetation types: cerrado denso (c.denso), cerrado sensu stricto (c.s.s), and campo sujo (c.sujo) over 30-45 day experimental period. The initial number of seeds was 120 for each vegetation type. Data points are means of 12 replicates of 10 seeds each. Error bars not shown.



Chapter 3

Dynamics of soil seed banks in tropical savannas of central Brazil (cerrado)

The coexistence of woody and herbaceous species (mainly grasses) is a fundamental feature of savanna ecosystems (Sarmiento 1984; Gardner 2006). Recent studies (Jackson et al., 1999; Higgins et al., 2000; Goldstein et al., 2008) have emphasized the importance of environmental variability and stochasticity in tropical savanna ecosystems to explain the coexistence of woody and herbaceous species. These studies recognize that savannas may not represent a stable (equilibrium) mixture of these life forms as has been suggested in other studies (Walker et al., 1981; Langevelde et al., 2003), but an unstable (non-equilibrium) mixture which persists as a result of complex, non-linear interactions between climate, soil, and disturbances such as fire, herbivory, and fluctuating annual rainfall (Goodland 1971; Goodland and Pollard 1973; Furley and Ratter 1988; Ratter et al., 1997; Furley 1999; Scholes and Archer 1997; Gardner 2006).

Studies on the early stages of the life cycle of woody species have often been ignored in the discussion of tree and grass coexistence and equilibrium models of tropical savannas. These early stages may affect not only the relative abundance of trees and grasses but also may determine the spatial distribution of these contrasting life forms (Hurtt and Pacala 1995). Thus, understanding equilibrium (or lack of equilibrium) conditions and coexistence between herbaceous and woody plants in savannas requires studies of the early stages of the life cycle of plant species such as seed dispersal, seed germination, and soil seed bank dynamics. In this study I will focus mostly on the early stages of the life cycle of woody species.

Soil seed banks play an important role in the long-term survival of individuals (Simpson et al., 1989; Baskin and Baskin 1998) as well as in the coexistence of plant species at a particular site because they constitute a source of propagules for recruitment after disturbances (Dalling and Hubbell 2002; Williams et al., 2005), and an alternative source of propagules when seed production of species fails for long periods. This reduction in seed production may occur as a result of interspecific competition or environmental heterogeneity (Chesson 1986; Baskin and Baskin 1998). Provided that seeds arrive from plants mostly growing in an immediate area, the soil seed bank composition should mirror the composition of the existing vegetation (Saulei and Swaine 1988). However, not all species in a community are necessarily represented in the seed bank (Amiaud and Touzard 2004; Wills and Read 2007; Hopfensperger 2007; Chaideftou et al., 2009). Disturbances and fragmentation, for example, may influence species richness and abundance in the soil seed bank (Young et al., 1987; Grombone-Guaratini and Ribeiro-Rodrigues 2002). In a Hawaiian forest exposed to both natural and anthropogenic disturbances, native seed plants composed 95% of the relative cover in the vegetation, and 99% of the seed rain composition, but only 33% of the soil seed bank (Drake 1998). Soil seed bank densities also can be influenced by rates of seed predation and pathogen infection in the soil, by intrinsic rates of loss in viability following dispersal, and by variation in the timing and duration of fruit production (Dalling et al., 1997; Dalling 2004; Fornara and Dalling 2005).

In the savannas of central Brazil (cerrado) the relative dominance of herbaceous and woody species varies along shallow topographic gradients of a few hundred meters length and approximately 30 m elevation. The upper portions of the gradients are characterized by closed savannas with a nearly continuous layer of trees and a relatively low abundance of herbaceous plants, while the lower portions of the gradient are characterized by open savannas with a continuous herbaceous layer and few isolated small trees or shrubs. (Eiten 2001). Differences in fire frequency alone cannot explain the tree density gradient because even after decades of fire suppression, the same relative difference between trees and herbaceous plant abundance is maintained along the gradient (Goodland and Pollard 1973; Furley and Ratter 1988; Furley 1999; Moreira 2000).

Cerrado species exhibit variations in flower and fruit production that are apparently determined by seasonal variations in precipitation and humidity (Lenza and Klink 2006). Studies also have reported seasonal variations in seed dispersal of herbaceous and woody species with a peak during the late dry and early wet seasons (Gouveia and Felfili 1998; Lenza and Klink 2006; Salazar unpublished results). Thus, timing of seed dispersal appears to be an important mechanism controlling timing of germination of cerrado species. However, seeds of many cerrado trees are dormant suggesting that dormancy could also control timing of germination by ensuring germination only under appropriate conditions (Sautu 2004).

Little is known about the dynamics of the soil seed bank and the characteristics of seeds of woody species in the cerrado. In particular, how well the soil seed bank composition reflects the vegetation species composition is unclear. In this study I tested the following hypotheses: (1) cerrado woody species form persistent soil seed banks from which seedlings recruit; (2) the number of species and density of seeds in the soil bank is higher in closed than in open savannas; (3) seeds dispersed during the dry season are long-lived, dormant, and have low moisture content, whereas those dispersed during the wet season are short lived, non-dormant, and have high moisture content; and (4) species composition of the soil seed bank, seed rain, seedling banks, and aboveground vegetation are similar.

Methods

Study site

This study was conducted from June 2005 until July 2008 at the Instituto Brasileiro de Geografia e Estatística (IBGE) reserve a field experimental station located 35 km south of Brasilia, Brazil (15˚ 56' S, 47˚ 63' W, altitude 1100 m). Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July, and August are often completely rainless. Average relative humidity during the rainy season is about 80% and decreases to 55% during the dry season when daily minimum relative humidity may reach values as low as 10%. Mean monthly temperatures range from 19˚C to 23˚C. The soils are very deep, well-drained, red or yellow, clay-rich oxisols; structurally strong but nutrient poor. The IBGE reserve contains all the major vegetation types: cerrado denso, cerrado sensu stricto, and campo sujo. Cerrado denso is a semi-closed woodland (40-60% tree crown cover) with a moderately tall (usually 7 m) tree canopy. Because of the shade created by the canopy trees, the herbaceous and semi-woody ground vegetation has a lower cover than in relatively open cerrado vegetation. Cerrado sensu stricto is a savanna dominated by trees and shrubs (canopy generally less than 7 m tall) with 10 to less than 40% tree crown cover. Campo sujo has few short trees and scattered shrubs over a nearly continuous grass layer (the woody layer usually covers less than 10% of the ground).

In June 2005, I established three transects (of approximately 1000 m) in the reserve, and each spanned the three major vegetation types. Transects were placed along continuous topographic gradients with different elevations (ranging from 1117 to 1153 m), and vegetation types that had been protected from fire for at least 30 years. I performed preliminary species-area curves in cerrado denso and campo sujo areas that indicated that about 85% of the maximum richness was attained at 1024 m^2. Nine plots of 400 m^2 (20 x 20 m) were established in each transect (three in each vegetation type), for a total of 27 permanent plots.

Soil seed bank

Seed bank composition and density were assessed through seedling emergence and therefore this study examines the germinable soil seed bank. To assess soil seed bank composition and density I collected soil samples in each of the 27 plots during late wet seasons (March 2006 and February 2007) and middle dry seasons (June 2006 and June 2007). During each collection period, soil samples were taken from 81 points (3 random points per plot) to a depth of 5 cm. This depth was chosen because preliminary studies found that 90% of the seeds were concentrated between 0.5 cm and 2 cm (Andrade 2002). At each point a total volume of 3,125 cm^3 of soil was extracted by using an iron square (25 x 25 x 5 cm) pushed into the soil. Within 24 hours of collection, the soil was passed through a sieve (5 mm) and large pieces of rock, roots, and litter were discarded. Each sample was then spread in plastic trays (25 x 30 x 4cm) which had several holes of

0.5 mm to allow drainage. Trays were placed in a shaded greenhouse and were watered daily with overhead sprinklers, keeping the soil constantly wet. Seed germination was checked every 4 days and seedlings were identified, counted and removed from the trays. Each tray was observed for 12 weeks. Individuals too small to identify at the end of 12 weeks were transplanted and grown to a larger size until they could be identified. To estimate the number of species that entered, left or persisted in the soil seed bank in each vegetation type, I counted the number of new, species, absent and persisting (re-occurring) species, as compared to the previous soil sampling season.

Seed characteristics of woody species

Fourteen woody species were used to test if seed moisture content, dormancy, and longevity differ between seeds dispersed during dry and wet seasons. These species are common and widely distributed throughout much of the cerrado (Silva Júnior 2005). They differ in their dispersal season and belong to a wide range of plant families: *Acosmium dasycarpum* (Fabaceae), *Brosimum gaudichaudii* (Moraceae), *Eriotheca pubescens* (Malvaceae), *Eremanthus glomerulatus* (Asteraceae) *Erythroxylum suberosum* (Erythroxylaceae), *Guapira graciliflora* (Nyctaginaceae), *Guapira noxia* (Nyctaginaceae), *Kielmeyera coriaceae* (Clusiaceae), *Miconia ferruginata* (Melastomataceae), *Ouratea hexasperma* (Ochnaceae), *Palicourea rigida* (Rubiaceae), *Rourea induta* (Connaraceae), *Solanum lycocarpum* (Solanaceae) and *Styrax ferrugineus* (Styracaceae).

During dry and wet seasons, available mature seeds from several individuals were collected directly from parent trees or from the ground if they were in good condition.

After mixing and cleaning, three groups of 50 seeds were weighed to calculate the average number of seeds per gram and also to determine fresh moisture content. To determine fresh seed moisture content the seeds were weighed, then dried at 70^{o} C for at least 72 hours, and then re-weighed until they attained a constant dry mass.

To evaluate natural seed dormancy I performed germination tests without any seed pre-treatment under greenhouse conditions. Five replicates of 20 seeds per species were sown in plastic trays (25 x 30 x 4cm) filled with an artificial substrate (mix of 70% peat moss and 30% vermiculite). The trays were randomly distributed in the greenhouse benches, under temperature conditions of 25-28ºC, 30% full sunlight, and watered continuously (three times a day for 2 minutes) with an automated sprinkler system. Germination, defined as radicle emergence, was monitored every three days for 8-12 weeks. I recorded the dates of the first and last seeds to germinate and calculated the median length of germination (pooling individuals of each species from the four replicates) from the germination times of all seeds which germinated (Blakesley et al 2002). A seed was considered dormant if the median length germination time (MLG) was 30 days or longer (Sautu et al., 2007).

Five different classes of dormancy were considered following the classification of Baskin and Baskin 2004: 1) physical dormancy (PY), species with a water impermeable seed (or fruit) coat; 2) morphological dormancy (MD), species with an underdeveloped embryo and median length germination (MLG) ≤30 days; 3) morphophysiological dormancy (MPD), those with an undeveloped embryo and MLG>30 days; 4) physiological dormancy (PD), those that have a permeable seed coat, fully-developed embryo and MLG>30 days; and 5) combinational dormancy (PY + PD), species with an impermeable seed coat and a physiologically dormant embryo.

A seed was considered to have physical dormancy if it did not imbibe water. The method described by Bansal et al. (1980) was used for the imbibition test. Briefly, seeds were weighed and then placed in water at room temperature (ca. 22º C). At hourly intervals for 8-24 hr, seeds were removed from the water, blotted dry, and reweighed. An increase in seed mass indicates that seeds (or fruits) have water-permeable coats, whereas no increase in mass indicates that they have water-impermeable coats. To determine whether seeds had a fully developed or underdeveloped embryo, I examined the embryos under a dissecting microscope after being removed from previously imbibed seeds.

Seed longevity was calculated using information on monthly viability loss by seeds kept stored in paper bags at room temperature and 40% relative humidity. Because seeds may experience drought periods of different length in field, I evaluated the rate of seed viability loss under laboratory dry storage conditions. Every month, for four-five months, seed viability percentage of each species was estimated by using 1% Tetrazolium red® solution (2, 3, 5-Triphenyltetrazolium chloride). Three samples of 50 seeds were used for each species every month. Seeds were soaked in the Tetrazolium solution in flasks totally wrapped with aluminum foil, which were placed in an oven and kept at 40°C. Embryo coloration patterns were evaluated under a dissecting microscope every hour until embryos were completely stained. For the species in this study, staining required 12 to 24 hours. Seeds were recorded as viable when embryos were homogeneously stained (i.e., both radicle and cotyledons). Exponential and linear functions, fitted to the decrease in viability loss as a function of time, were used to predict the viability of each species after 12 months of storage.

Leaf area index, species diversity and relative abundance of woody plants

During the dry season of 2005 I estimated leaf area index (LAI: area of leaves per unit area of soil surface) using a Decagon® AccuPAR model LP-80 PAR/LAI Ceptometer). This instrument calculates LAI based on the above and below-canopy photosynthetic photon flux density (PPFD: the flux of photons between 400 and 700nm wavelength per unit area) measurements. LAI measurements were taken 1m above the soil surface, at midday (on clear days) at 8 random points within each plot.

Species composition and abundance in each plot was assessed by identifying and counting all woody individuals taller than 1 m. For each plot, within each vegetation type I calculated Shannon diversity index (H'): $H' = - \Sigma\, p_i \, ln p_i$, and Dominance index D_o: $D_o = 1\text{-}D$; p_i is the proportion of species i relative to the total number of species, and D is Simpson diversity index.

Seed rain and seedling bank

Species composition, abundance and diversity of seed rain at the study sites were estimated from data collected in a previous study (Salazar, unpublished results) using seed traps located at the corners of each of the permanent plots (4 per plot). Seed traps consisted of a plastic funnel, 20 cm in diameter, with a collection area of 0.0314 m^2 that was inserted into a PVC tube (5 cm diameter) that held the funnels in place. A small nylon mesh bag was attached at the funnel base to collect seeds and dispersal units. All seeds, fruits, and fruit parts were collected once a month for a 13- month period, from November 2005 until February 2007. Whole, apparently intact seeds/diaspores were identified to species (or at least to family when it was not possible to identify the species) and dried to constant weight. Identification was based on published literature and by comparisons with herbarium specimens and specimens collected from mature plants.

To determine seedling bank composition, in July 2005 eight $1m^2$ subplots were randomly established within each of the 27 permanent plots for a total of 216 subplots. According to preliminary curves of richness of seedling species versus number of $1m^2$ subplots performed in cerrado denso and campo sujo areas, about 90% of the seedling richness was attained with eight $1m^2$ subplots. In July 2006, all seedlings of woody species up to 30 cm tall within the subplots were counted and identified. Identification was made by comparison with herbarium specimens and with reference specimens collected from outside the subplots and with help from local botanists. Sixteen percent of seedlings could not be identified.

Data analysis

Differences in the number of species/m^2 and seed density of the germinable soil bank among vegetation types and collection periods (season) were examined with two-way ANOVAs with vegetation type and season as main fixed factors. To fulfill the assumption of normality, the data of seed density were square- root transformed. Non-parametric Kruskal-Wallis tests were used to test differences in the proportion of woody/herbaceous species among vegetation types and among seasons as well as differences in species diversity (Shannon index) among vegetation types. Negative exponential and linear functions were fitted to the data of percentage seed viability loss as a function of time. Differences in moisture content and longevity between seeds dispersed in dry and wet seasons were examined with student's *t* test.

Differences in species abundance, diversity and leaf area index among vegetation types were examined with one-way ANOVAs using the program JMP 7 (SAS Cary, NC). The data from the nine plots of each vegetation type were pooled. Tukey HSD post hoc tests were used for multiple comparisons of means.

Non-metric multi-dimensional scaling (NMDS) ordination was used to examine patterns in species composition. Ordinations for the vegetation, seed rain, soil seed bank and seedling bank of woody species were performed with PRIMER v5 (Clarke & Warmick 2001) combining all taxa found during the sampling periods (in the case of seed rain and seed bank). The purpose of the ordinations is to represent the sites as points in a two dimensional space such that the relative distances among points reflect the relative dissimilarities of the sites. Similarities between every pair of the nine sites were calculated using Bray-Curtis coefficients, after square-root transforming species abundances of every entry in the similarity matrix.

Analyses of similarities in species composition among vegetation types were examined with an analogue of the 1 way-ANOVA (ANOSIM) under the null hypothesis that there are no species assemblage differences among vegetation types. ANOSIM routines are permutation/randomization tests that assess differences between average ranks between and within groups. Values on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity between groups) reflect multiple pairwise comparisons with groups well separated ($R > 0.75$), groups overlapping but differing (R> 0.5) or barely separated at all (R <0.25). To determine the extent of agreement between pairs of ordinations (agreement in the high-dimensional multivariate data underlying this low dimensional plot), spearman rank correlation coefficients (ρ) between all the elements of any two similarity matrices were calculated under the null hypothesis that there is no relation whatsoever between any two similarity matrices (ρ will be 0).

Results

Soil seed bank density

The germinable soil seed bank collected in all study sites between March 2006 and July 2007 contained 35 species belonging to 25 genera and 10 families (Table 3.1). Density of herbaceous and woody species seeds over the four collection periods was higher in cerrado denso (577.75seeds/m^2) than in campo sujo (386.62 seeds/m^2) or cerrado sensu stricto (276.81 seeds/m^2). Seeds of shrubs and herbs of Asteraceae, Poaceae, Rubiaceae, Cyperaceae and Melastomataceae were the most abundant and only ten woody species (trees and shrubs) were found in the soil seed bank during the period of study (Table 3.1).

Vegetation type (2 way anova $F_{2,312} = 0.17$; $p = 0.84$), season ($F_{3,312} = 2.20$; $p = 0.08$ and their interaction ($F_{6,312} = 1.61$; $p = 0.14$), did not have a significant effect on the number of species per m^2 found in the germinable soil seed bank (Figure 3.1A). Except for the dry season of June 2007, the mean number of species tended to be higher in cerrado denso (the vegetation type with highest leaf area index) than in cerrado sensu stricto or campo sujo (Figure 1a). Vegetation type ($F_{2,312} = 3.38$; $p = 0.03$) and season (F

$_{3,312} = 4.39$; $p = 0.005$), but not their interaction ($F_{6,312} = 1.21$; $p = 0.30$), had a statistically significant effect on soil seed density (Figure 3.1.B) indicating that seasonal changes in soil seed bank density did not depend on vegetation type. Mean seed density was significantly higher in cerrado denso (144.30 ± 24.13) than cerrado sensu stricto (70.20 ± 10.91). Seed density in campo sujo (98.22 ± 21.66) did not differ from that in cerrado denso or cerrado sensu stricto. Seed density was significantly higher in the dry season of June 2006 than in the wet season of 2007, and did not differ significantly among the other seasons. The proportion of woody/herbaceous species did not differ significantly among vegetation types ($H = 0.4351$; $p = 0.804$) but differed significantly among seasons ($H= 9.264$; $p = 0.026$) with a higher proportion of woody/herbaceous species during the wet season of 2006 than during other sampled seasons (Figure 3.1.C).

Soil seed bank persistence

Less than 16% of all the herbaceous and woody species found in the soil were present in the four sampling seasons suggesting that they were among the few species forming persistent seed banks (seeds living in the soil for a period longer than one year). Only *Baccharis salzmanni, Echinolaena inflexa and Miconia albicans,* were consistently present in all vegetation types. *Spermacoce c.f verticilata* and *Cassitha filiformis* were found in all four sampling periods but only in cerrado sensu stricto and campo sujo. More than 48% of the species were present in only one of the four sampling seasons. Similar trends in the number of species entering, exiting and persisting in the soil seed bank were observed in each vegetation type over the period of study (Figure 3.2) with more species leaving the soil bank during the wet season of 2007 than during the dry seasons of 2006 and 2007. In the wet season of 2007, the number of species leaving the soil bank was higher than both the number of species entering into the bank and the number of species persisting in the bank, in all three vegetation types. The number of species persisting in the soil bank was higher during the dry seasons than during the wet season of 2007 (Figure 3.2).

Seed characteristics of woody plants

The number of seeds per gram of woody species ranged from 3.31 to 654.04 (Table 3.2). Seed moisture content ranged from 8.91% to 81.62%. Nine of the 14 species (64.28%) had moisture content higher than 28% (moist seeds), and five species (35.7%) had less than 28% (dry seeds) moisture content. Dispersal season had a significant effect on seed moisture content ($t_{1,34} = 5.38$; $p < 0.001$); species dispersed in the dry season had lower moisture content (22.49% ± 4.98) than those dispersed in the wet season (59.36% ± 4.62) (Table 3.2).

I assigned seed dormancy types based on median length germination time (MLG), permeability of the seed coat to water and type of embryo. Seeds of *Eremanthus glomerulatus* did not germinate probably because of extremely low initial viability (2.47%). Six species (42.86%) were non-dormant (MLG ≤ 30 days) and seven species (57.14%) were dormant (Table 3.2). Seventy five percent of the species dispersed during the dry season had dormant seeds whereas 43% of the species dispersed during the wet season had dormant seeds (Table 3.2). *Acosmium dasycarpum* was the only species exhibiting physical dormancy (PY) because of impermeable seed coats (data not shown). Four species (28.57%) had permeable coats and MLG> 30 days and thus were classified as having physiological dormancy (PD). Lastly, two species (14.29%) were assigned to have morphophysiological dormancy (MPD) because they exhibited permeable coats, underdeveloped embryos, and MLG > 30 days (Table 3.2). No species were found to have either morphological dormancy (MD) or combinational dormancy (PY + PD)

Seed longevity ranged from 5.81 months to 26.48 months; with only three species (25%) maintaining their viability longer than 1 year (Table 3.2). Dispersal season did not have a significant effect on seed longevity ($t_{1,\ 34}$ =0.55; p =0.29); however seeds of species dispersed in the dry season lived slightly longer (12.72 ± 4.95 months) than those dispersed in the wet season (10.32 ± 2.34 months) (Table 3.2).

LAI, species diversity and relative abundance of woody plants

The major difference in vegetation structure among the vegetation types was in leaf area index (LAI) which ranged from about 1.5 in cerrado denso to about 0.6 in campo sujo (Figure 3.3A). Species diversity was significantly higher in cerrado denso than in cerrado sensu stricto (Kruskal-Wallis test, H=12.17; p=0.0005) or campo sujo (H=5.47; p=0.02 Figure 3.3B). Species diversity did not differ between cerrado sensu stricto and campo sujo (Kruskal-Wallis test H=0.56; p=0.45; Figure 3.3B). Mean abundance (number of 1 m tall individuals in 400m^2) did not differ significantly among vegetation types (Figure 3.3C). The total number of species found in all study plots also was higher in cerrado denso than in campo sujo or cerrado sensu stricto (Table 3.3).

Some species with relative abundance above 2% were well represented in the three vegetation types, but many others had abundances lower than 2% and therefore are not shown in Figure 3.4. *Miconia albicans* was well represented in all vegetation types, and especially in cerrado sensu stricto. In contrast, *Baccharis salzmanni* was a dominant species in campo sujo and cerrado sensu stricto, but not in cerrado denso (Figure 3.4). Likewise, *Blepharocalix salicifolius* was the most abundant species in cerrado denso, but in cerrado sensu stricto and campo sujo this species had a low relative abundance (less than 2%) (Figure 3.4). The distribution of the relative abundance of woody species in the three vegetation types exhibited a reverse J shape; however, this shape was more pronounced in campo sujo than in cerrado sensu stricto or cerrado denso. Mean dominance index was significantly lower (0.05 ± 0.003) in cerrado denso than in cerrado sensu stricto (0.16 ± 0.02) or campo sujo (0.14 ± 0.02).

Seed rain and seedling bank

The total number of species found in the annual seed rain ranged from 23 in campo sujo to 28 in cerrado sensu stricto (Table 3.3). Although the total number of propagules per m^2 was lower in campo sujo than in cerrado denso or cerrado sensu stricto, the Shannon diversity index was slightly higher in this vegetation type than in cerrado sensu stricto or cerrado denso (Table 3.3). The total number of woody species found in the seedling bank in cerrado sensu stricto was lower than in cerrado denso or campo sujo. Seedling density, however, was slightly higher in cerrado denso than in campo sujo. In general, species diversity measured by the Shannon index was lower in cerrado sensu stricto than in cerrado denso or campo sujo respectively (Table 3.3).

Similarity in species composition among seed bank, seed rain, seedling bank, and standing vegetation

Species composition of woody plants in the nine study sites differed among vegetation types (Figure 3.5.a). Sites from each of the three vegetation types partially overlapped in their species composition but were statistically distinguishable in the 2-dimensional ordination space (ANOSIM; $R = 0.66$, $p = 0.007$). On average, cerrado denso sites were more similar to each other (shorter distances in the ordination space) than cerrado sensu stricto sites or campo sujo sites. The average similarity of the three sites of cerrado denso (average of the three pair wise comparisons) was 66.40%; of cerrado sensu stricto 62.06%; and of campo sujo 59.98%. On the other hand, the soil seed bank composition of woody species did not differ significantly among the vegetation types (Figure 3.5b). Consequently, the soil seed bank composition of the three vegetation types barely separated in the two dimensional plot (ANOSIM; $R = 0.128$, $p = 0.268$). The average similarity in species composition of the seed bank composition of campo sujo sites was 61.39%; cerrado sensu stricto sites 48.72%; and cerrado denso sites 46.68%.

Composition of woody species in the seed rain differed among vegetation types (Figure 3.5.b) (ANOSIM; $R = 0.49$, $p = 0.014$). Seed rain composition of cerrado sensu stricto sites was more similar to each other than seed rain composition of cerrado denso and campo sujo sites. The average similarity of cerrado sensu stricto sites was 49.31% versus 45.76% for cerrado denso sites and 42.96% for campo sujo sites.

Species composition of the woody seedling bank differed significantly among vegetation types (ANOSIM; $R = 0.753$, $p = 0.0004$) and the sites separated well from each other in the ordination space (Figure 3.5.d). Seedling composition of cerrado sensu stricto sites was more similar to each other than to that of cerrado denso and campo sujo sites. The average similarity of the three sites of cerrado sensu stricto was 73.38% versus 71.99% for cerrado denso sites and 69.36% for campo sujo sites.

Seed rain and vegetation composition across sites were more similar to each other than either was to the seed bank and seedling bank composition of woody species (Table 3.4). The correlation between the vegetation and the seed rain was 0.52. Seedling bank composition was more similar to the vegetation composition than to the seed bank or seed rain (Table 3.4). The correlation between the seedling bank and the vegetation was 0.29; between seedling bank and seed bank 0.12; and between seedling bank and seed rain, 0.03.

Discussion

Soil seed bank persistence

More than 50% of the herbaceous and woody species found in the soil seed bank in each vegetation type were present only during one of the four consecutive sampling periods which suggests that most species of the soil seed bank form very short-lived, transient banks. Sixteen percent of woody and herbaceous species found in the soil at each vegetation type were observed in all four seasons suggesting that these species, which represent a small percentage of all reproductive plants in the area, might form persistent banks. Among the 10 woody species found in the soil seed bank, only *Baccharis salzmanni* and *Miconia albicans* were well represented in all four collection periods (seasons), indicating that, with the exception of these two species, and contrary to our initial hypothesis, most cerrado woody species do not form persistent soil seed banks. This low persistence among woody species could be explained by their short seed longevity. Among the 14 woody species studied, only four species maintain their viability for periods longer than 12 months (at levels lower than 15%).

It is possible that many cerrado species maintain their seeds inside fruits rather than in the soil. Fruits of several cerrado woody species exhibit long periods of development and maturation times that range from 3 to 12 months (Oliveira and Silva 1993; Oliveira 1998) after which most seedlings establish in the rainy season (Gottsberger and Silberbauer- Gottsberger 2006). This apparent adjustment of fruit maturation and seed dispersal times could be the result of selective pressures to minimize seed damage and maximize seedling establishment in environments subjected to disturbance such as high fire frequency. For example, some fire-adapted species retain long-lived dormant seeds stored in non-deciduous fruits on the plant themselves from which they are released by exposure to fire (Fenner 1985; Baskin and Baskin 1998). Other disturbances such as changes in nutrient availability, and fluctuations of the water table depth, could impose similar selective pressures in cerrado woody species for enhancing the retention time of their seeds on parental plants rather than in soils.

Soil seed bank density

Total soil seed density of herbaceous and woody species during the study period was higher in cerrado denso than in campo sujo and cerrado sensu stricto. Soil seed bank in the three vegetation types mostly comprised herbaceous species and very few woody species (Table 3.1). The relatively high abundance of herbaceous species found in the seed bank has been previously reported for cerrado (Andrade 2002) and for other tropical savannas (McIvor and Gardener 1991; Perez and Santiago 2001). In our study, mean density of seeds in the soil ranged from 144.30 seeds/m^2 in cerrado denso, to 70.20 seeds/m^2 in cerrado sensu stricto. This is within the range of 25 to 3,350 seeds per m^2 reported for tropical forests (Garwood 1989), and it is also within densities reported in other cerrado studies (i.e., 757 seeds m^2, Sassaki et al., 1999; 143 seeds m^2, Andrade 2002) for soil samples obtained from the first 5 cm depth of soil. The average soil seed density of the three vegetation types (104.38 seeds m^2) in this study, differed from seed densities observed in tropical savannas of Venezuela (1.70 seeds m^2, Perez and Santiago 2001; 52.50 seeds m^2, Flores and Dezzeo 2005). Seed bank densities in the three vegetation types studied also were lower than seed densities found in burned and tilled soils, and in areas planted with pasture that were covered by cerrado vegetation in the past (Andrade 2002; Ikeda et al., 2008); in these areas, seeds of non-native species formed larger seed banks than seeds of native species. In our study, I found few non-native species such as *Ageratum conyzoides*, *Achrynoline satureoides*, and *Spermacoce cf vertlicilata* in the soil, probably because of the lack of recent major disturbances (i.e., fire).

Contrary to our hypothesis the mean number of species per m^2 in the soil bank did not differ among vegetation types, however the total number of species per m^2 in the soil bank across collection periods was slightly higher in closed than in intermediate and open savannas, respectively (Table 3.1). Vegetation type and season, on the other hand, had a significant effect on soil seed density, suggesting that seasonal changes in soil seed bank density occurred independently of vegetation type effects or that the relative changes in soil seed density were similar. Soil seed density was higher during dry seasons than during wet seasons for all three vegetation types. This pattern also was consistent with the high number of species persisting in the soil during dry seasons. Seeds could persist better in the soil during the dry season because clay- rich soils tend to develop prominent cracks, of varying depth and width, where seeds easily can become buried (Garwood 1989; Espinar et al., 2005). In contrast, during the wet season heavy rains can fill the spaces between soil aggregates, limiting opportunities for small seeds to incorporate into the soil (Pearson et al., 2003; Dalling 2004).

Differences in soil seed density between dry and wet seasons also could be the result of the phenology of seed dispersal and germination in the cerrado. In another study (Salazar, unpublished results) I found that in all three vegetation types more seeds were dispersed during the mid and late dry season and early wet season, in particular wind-dispersed species. Most cerrado woody species seeds germinate early in the wet season (Oliveira and Silva 1993;Gottsberger and Silberbauer- Gottsberger 2006); Thus, soil collected during the mid dry seasons (June 2006 and June 2007) could have a higher number of seeds (that were dispersed in the previous season) than the soil collected during the late wet seasons (March 2006 and February 2007), after the germination season was over.

I found that wind dispersed-species belonging to the Asteraceae, Cyperaceae and Poaceae were well represented in the seed bank (Table3.1). Some members of these families have been reported forming persistent seed banks in other studies (Baskin and Baskin 1998). In contrast, animal-dispersed seeds in this study were not well represented in the seed bank (except for *Miconia albicans*). Because fruits of these species contain a relatively high amount of fleshy tissues that attract animal dispersal agents, it is likely that processes such as secondary dispersal and predation are more likely fates for seeds of these species rather than their passive incorporation into the soil (Dalling 2004). Wind-dispersed seeds, on the contrary, could be less palatable to animals and hence relatively better at incorporating into the soil. Finally, the differences in seed density found between the two dry seasons and between the two wet seasons could be attributed to differences in annual seed rain and/or differences in mean annual precipitation (4.6 mm in 2006 versus 3.2 mm in 2007; IBGE-Recor metereological data:http://www.recor.org.br).

Seed characteristics of woody species

I found that seed moisture content, dormancy, and longevity of cerrado woody species were consistent with dispersal season. Seeds dispersed during the dry season had, on average, lower moisture content, slightly higher longevity, and higher percentage of dormant seeds than those dispersed during the wet season. The consistency between these seed characteristics and dispersal season suggests that timing of seed dispersal is highly involved in controlling timing of germination in cerrado species so that germination occurs when environmental conditions are suitable for seedling establishment (Fenner and Thompson 2005). Previous studies in the cerrado have shown that non-dormant seeds and seeds coming out of dormancy at the beginning of the wet season can germinate immediately after the first heavy rains once soil moisture becomes a non-limiting factor in the upper soil layers (Gottsberger and Silberbauer-Gottsberger 2006). In this study, the proportion of species with dormant vs. non-dormant seeds was relatively higher during the dry season than during the wet season. Thus, it seems that in addition to timing of dispersal, dormancy also is involved in controlling timing of germination. Although I have data for only a few species, our results suggest that similar to tropical forests, time of germination of cerrado species could be controlled by both seed dormancy and timing of dispersal (Garwood 1983; Sautu et al., 2007).

Seeds dispersed during the dry season were wind-dispersed (except for *E. suberosum*,) while seeds dispersed during the wet season were mostly animal-dispersed. The ability of seeds to survive desiccation has been related to their initial moisture content. Unlike desiccation sensitive seeds, desiccation tolerant seeds remain viable at low moisture contents and tolerate subsequent rehydratation without significant loss of viability (Roberts 1973). The low moisture content of seeds dispersed during the dry season, in our study, coupled with their wind-dispersal mode could enable them to tolerate extended desiccation periods until the beginning of the wet season. Moist seeds dispersed during the wet season (or shortly before) of cerrado woody species, on the contrary, could not tolerate long desiccation periods.

Similarity in species composition among seed bank, seed rain, seedling bank and standing vegetation

Although tree density of woody species taller than 1 m did not differ significantly among the vegetation types, species diversity, composition and canopy cover (measured as leaf area index) of woody species differed significantly among the three vegetation types. Cerrado denso was more species diverse and exhibited higher canopy cover than cerrado sensu stricto and campo sujo which were dominated by relatively few woody species. I found that vegetation and seed rain were moderately similar to one another in their species composition. In contrast, the species composition of the germinable seed bank differed greatly from that of both the vegetation and the seed rain. The relatively high similarity between the seed rain and the vegetation composition (Table 3.4) suggests that seeds of a large number of species fall near parental trees, and that long distance dispersal contributed few seeds of species not growing nearby. However, it is possible that animal-dispersed seeds of some woody species were not captured in our seed traps. Wind- and gravity-dispersed seeds usually are better represented in seed traps than are animal dispersed seeds (Kollmann and Goetze 1998).

The composition of the germinable seed bank differed from that of the vegetation and the seed rain largely because very few woody species were found in the soil seed bank. No assemblage differences in the woody species composition of the seed bank among the three vegetation types were observed. The low similarity between the seed rain and the seed bank composition, between the seed bank and the vegetation composition, and between the woody seedling bank and the seed bank composition, also suggest that recruitment from the soil seed bank is extremely low. Despite differences in seed rain input (i.e., seed rain density was lower in open savannas than in closed savannas), seed bank composition did not differ significantly among sites. As indicated above, this could be the result of the extremely short longevity of woody species seeds that prevents the formation of persistent seed banks.

Often, in tropical forests, there is little correspondence between the species composition of the vegetation and the seed bank (Hall and Swaine 1980; Young et al., 1987; Saulei and Swaine 1988; Hopfensperger 2009), or between the annual seed rain and the seed bank (Saulei and Swaine 1998). The same patterns have been found in temperate forests (Fenner and Thompson 2005), and grasslands (Peco et al., 1998). These dissimilarities have been explained by the minor contribution of long-lived species to the seed bank, and because in general, pioneer species contribute much more to the soil seed bank than do secondary species (Harper 1977). High seed predation and fungal pathogen infection after dispersal also could contribute to the dissimilarity between vegetation and seed bank composition by reducing the number of viable seeds buried in the soil. (Murray and Garcia 2002; Schafer and Kotanen 2003; Fornara and Dalling 2005). I think that most cerrado woody species do not form persistent soil seed banks (seeds living longer than one year in the soil) to ensure germination at the beginning of the rainy season soon after dispersal and thus guarantee the subsequent survival and establishment of seedlings throughout the seven month period (October-March) of the wet season. The strong seasonal variations in seed maturation and dispersal result in a higher proportion of non-dormant seeds falling on to the soil at the beginning of the rainy season ensuring seedling survival and establishment.

I found low similarity in species composition between woody plants and woody seedlings, and between woody seedlings and woody seed rain. These results further support the point of view that very few of the seeds that are locally dispersed become successfully established as seedlings. These results also could suggest high establishment limitation, the absence of recruitment because of limited availability of suitable sites that provide the conditions required for a particular seed to germinate and become established (Harper 1977; Muller-Landau et al., 2002).

The lack of persistent soil seed banks of woody species despite the presence of woody seedlings is consistent with some assumptions of the non-equilibrium, demographic-bottleneck models of tree-grass coexistence in savannas (Higgins et al., 2000; Sankaran et al., 2004; Meyer et al., 2007). These models represent a departure from traditional equilibrium models of savannas (e.g. trees and grasses have different rooting depth to avoid competitive exclusion) in that the emphasis is on demographic rather than physiological mechanisms of water and nutrient exploitation. For example, in arid savannas, the primary demographic bottleneck for trees is thought to be the germination and seedling establishment stage (Jeltsch et al., 1998). In mesic savannas, the death of saplings, because of frequent fires, is assumed to be the main demographic mechanism that prevents recruitment to adulthood (Higgins et al., 2000). In the savannas of Brazil, Gardner (2006) identified the differences in tree reproductive response to fire as the main determinant of tree-grass ratios across vegetation types. Our study indicates that in the absence of fire, the recruitment stage is also a significant demographic bottleneck for woody species because recruitment of cerrado woody seedlings occurs only when seed dispersal coincides with appropriate environmental conditions that allow rapid germination.

This study shows that during periods favorable to sexual reproduction, when major disturbance factors such as fire are excluded from the savannas: (1) the seed bank in all vegetation types was mostly composed of herbaceous species while most woody species did not form persistent seed banks; (2) density of seeds in the soil was higher in cerrado denso than in cerrado sensu stricto and campo sujo and therefore paralleled changes in LAI along topographic gradients; (3) most of the seeds dispersed during the dry season were wind-dispersed, exhibited low moisture content, and were dormant. In contrast, most of the seeds dispersed during the wet season were animal- dispersed, exhibited high moisture content and were non-dormant allowing them to germinate with the first heavy rains; (4) seed rain was mostly produced by local trees, and consequently short distance dispersal was an important source of diaspores in these cerrado ecosystems; (5) woody species composition of the soil seed bank and seedling bank largely differ from the vegetation, and the seed rain species composition, suggesting low recruitment of woody species from the soil seed bank, therefore, (6)seedling recruitment of cerrado woody species occurs in the rainy season when seed dispersal coincides with high soil moisture content.

Table 3.1. Mean seed density (number of seeds/m^2) of herbaceous and woody species found in the soil seed bank of each vegetation type (cerrado denso, cerrado sensu stricto and campo sujo) over four sampling periods. Soil samples were collected during two consecutive wet seasons (March 2006 and February 2007) and two consecutive dry seasons (June 2006 and June 2007). P: perennial, A: annual. Leaf phenology: Evergreen (E).Deciduous (D). *: not determined

Family	**Species**	**Growth form**	**cerrado denso**	**cerrado sensu stricto**	**campo sujo**	**Average**
Asteraceae	*Achyrocline saturoides*	Herb-P	0.30	0.59	0.15	0.35
	Ageratum conyzoides	Herb-A	0.15	0	0.15	0.10
	Morphospecies 1	*	0.30	0.15	0.15	0.20
	Morphospecies 2	*	1.19	0.15	0	0.44
	Baccharis salzmannii	Shrub-D	26.81	17.78	17.49	20.69
	Bidens pilosa	Herb-A	0.30	0.30	0.44	0.35
	Eremanthus glomerulatus	Tree-E	0	1.04	0.30	0.44
	Eremanthus goyazensis	Tree-E	0.30	1.04	0.74	0.69
	Eremanthus sp.	Tree-E	0.15	0	0	0.05
Cyperaceae	*Bulbostylis capilaris*	Herb-P	0.89	0.59	0	0.49
	Bulbostylis sp.	Herb-P	3.58	0.30	0.89	2.42
	Rhynchospora sp.	Herb-P	1.04	1.93	1.54	1.50
	Rhynchospora exaltata	Herb-P	2.52	1.19	4.00	2.57
Lamiaceae	*Hyptis cf lophanta*	Herb-A	0.30	0.15	0.15	0.20
Lauraceae	*Cassitha filiformis*	Herb-P	7.70	11.41	11.75	10.29
Fabaceae	*Chamaecrista cf. orbiculata*	Shrub-D	0.74	0.61	0.89	0.75
	Leguminosae 1	*	0.15	0.15	0	0.10
	Leguminosae 2	*	0.15	0	0	0.05
	Mimosa sp.	Tree-D	0.44	0	0.15	0.20
Malvaceae	*Sida rhombifolia*	Herb-P	0.44	0	0	0.15
Melastomataceae	*Miconia albicans*	Shrub-E	17.48	2.96	6.98	9.14
	Ossaea congestiflora	Shrub-E	0.59	0.15	0.16	0.30
Oxalidaceae	*Oxalis densifolia*	Herb-A	0.74	0.89	0.30	0.64
Poaceae	*Axonopus sp.*	Herb-P	0.15	0.30	0	0.15
	Echinolaena inflexa	Herb-P	76.09	23.26	43.20	58.65
	Ichnanthus camporum	Herb-P	0.15	0.44	0.15	0.25
	Morfospecies 1	*	0	0	0.15	0.05
	Morfospecies 2	*	0	0.15	0	0.05
	Paspalum cf. gardnerianum	Herb-P	1.63	0.89	1.27	1.26
	Schizachyrium tenerum	Herb-P	0.07	0.15	0.30	0.17
	Trachypogon sp.	Herb-*	0.15	0	0	0.05
Rubiaceae	*Coccocypselum aureum*	Herb-*	0.30	0	0	0.10
	Cordiera sp.	Tree	1.04	0	0	0.35
	Sabicea brasiliensis	Shrub	0.30	0	1.33	0.54
	Spermacoce cf verticilata	Herb-P	3.26	2.67	4.04	3.32
Total			144.30	70.20	98.22	104.38

Table 3.2. Seed characteristics of 14 woody species from the Brazilian cerrado. Dispersal season: Dry (D), Wet (W), Dry and Wet (DW). Seeds/g: number of seeds (or diaspore)/g. Seed MC: mean fresh moisture content ± SD. Dormancy type (abbreviations from Baskin and Baskin 2004): non dormant (ND), physical dormancy (PY), physiological dormancy (PD), morphophysiological dormancy (MPD)*. Longevity: number of months that seeds remain viable (% viability >1%) in dry storage conditions at 20°C. Viability at 1 year: Exponential and linear functions, fitted to the decrease in viability loss as a function of time, were used to predict the viability of each species after 12 months of storage. *: not determined.

Species	**Dispersal season**	**Seeds/g**	**Seed MC (%)**	**Dormancy**	**Longevity (months)**	**Viability at one year (%)**
Acosmium dasycarpum	D	25.08	9.78 ± 3.44	PY	13.43	9.25
Brosimum gaudichaudii	W	17.06	47.19 ± 4.96	ND	*	*
Eremanthus glomerulatus	D	654.04	9.44 ±1.96	*	*	*
Eriotheca pubescens	D	3.31	9.96 ± 0.46	ND	5.81	0.00
Erythroxylum suberosum	D	5.85	66.14±1.01	MDP	26.48	10.95
Guapira graciliflora	W	8.88	64.41± 0.29	ND	16.34	15.29
Guapira noxia	W	27.86	54.82 ± 0.91	ND	3.80	0.00
Kielmeyera coriaceae	D	9.01	24.36 ± 4.12	PD	5.18	0.00
Miconia ferruginata	W	18.61	71.22± 0.01	PD	7.56	0.00
Ouratea hexasperma	W	28.74	37.51±1.35	ND	9.01	0.00
Palicourea rigida	W	17.32	72.92 ± 0.77	PD	14.92	11.14
Rourea induta	W	5.31	67.47± 0.98	PD	--	--
Styrax ferrugineus	DW	4.59	81.62 ± 0.04	MDP	7.56	0.00
Solanum lycocarpum	DW	6.00	8.91 ± 1.44	ND	7.72	0.00

*No species showed morphological nor combinational dormancy

Table 3.3. Total number of species, density (vegetation: number of individuals/m^2; seed rain: number of diaspores/m^2; seedling: number of seedlings /m^2), and Shannon diversity indices of woody species found in the vegetation, seed rain and seedling bank in three vegetation types: cerrado denso, cerrado sensu stricto, and campo sujo.

	Vegetation type	**Number of species**	**Density**	**Shannon diversity index**
Vegetation	cerrado denso	90	0.78	3.69
	cerrado sensu stricto	76	0.86	2.93
	campo sujo	86	0.83	3.18
Seed rain	cerrado denso	24	976.65	2.23
	cerrado sensu stricto	28	1165.04	2.24
	campo sujo	23	484.78	2.37
Seedling	cerrado denso	45	4.64	3.26
	cerrado sensu stricto	36	4.40	3.10
	campo sujo	45	4.15	3.32

Table 3.4. Spearman rank correlation coefficients (ρ) between pairs of similarity matrices indicates how closely related a set of multivariate data are. In this case, the data are the abundance of each species present in the vegetation, seed rain, seed bank, and seedling bank. ρ = 1 if similarity matrices are identical. ρ = 0: no relation between similarity matrices. *P* values are indicated in parentheses

	Vegetation	**Seed rain**	**Seed bank**
Seed rain	0.52 (*p*= 0.0001)		
Seed bank	0.08 (*p*=0.654)	0.03 (*p*= 0.559)	
Seedling bank	0.29 (*p*=0.054)	0.03 (*p*= 0.451)	0.12 (*p*=0.24)

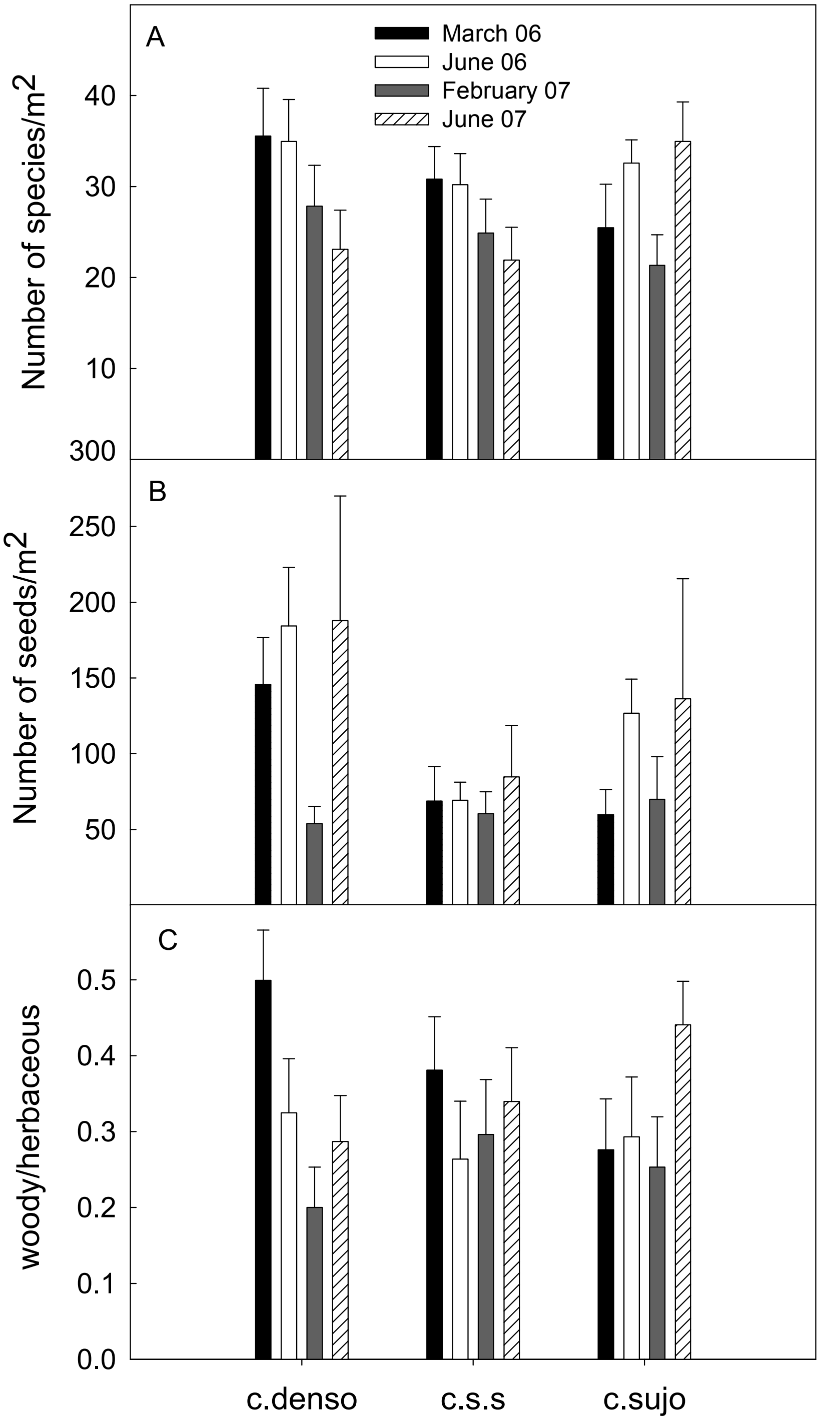


Figure 3.1. a) Number of species/m^2, b) number of seeds/m^2 (density), and c) proportion of woody/herbaceous species in the germinable soil seed bank collected during wet (March 2006, February 2007) and dry (June 2006, June 2007) seasons. Species richness and number of seeds include both herbaceous and woody species. Soil samples are from cerrado denso, cerrado censu stricto, and campo sujo (white bars) (n=27). Bars are means + SE (n=27).

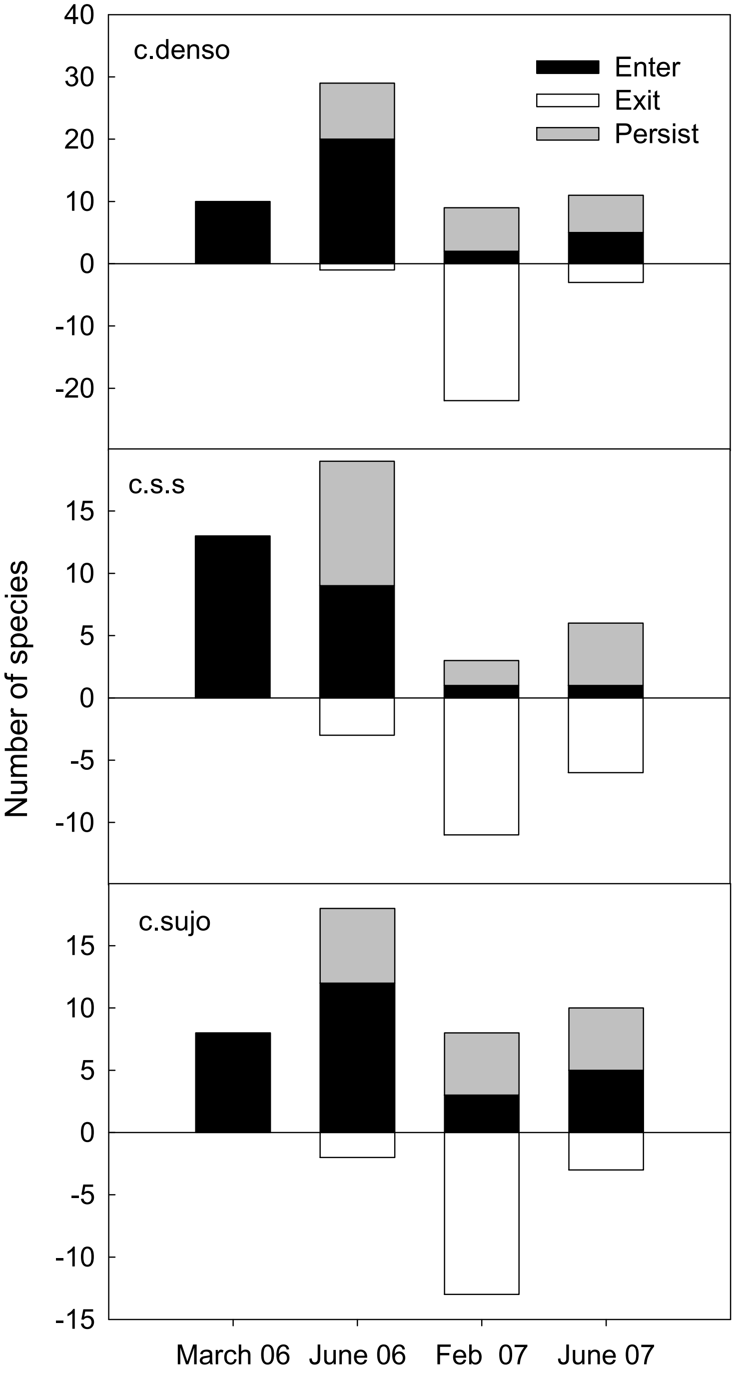


Figure 3.2. Number of species that enter (black), number of species that exit (white) and number of species that persist (gray) in the soil seed bank with respect to the previous collection period in each vegetation type from March 2006 until June 2007: cerrado denso (c. denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo). The bar of March 2006 indicates the number of species found in the soil bank at the beginning of the study. Soil samples were collected during two consecutive wet seasons (March 2006 and February 2006) and two consecutive dry seasons (June 2006 and June 2007).

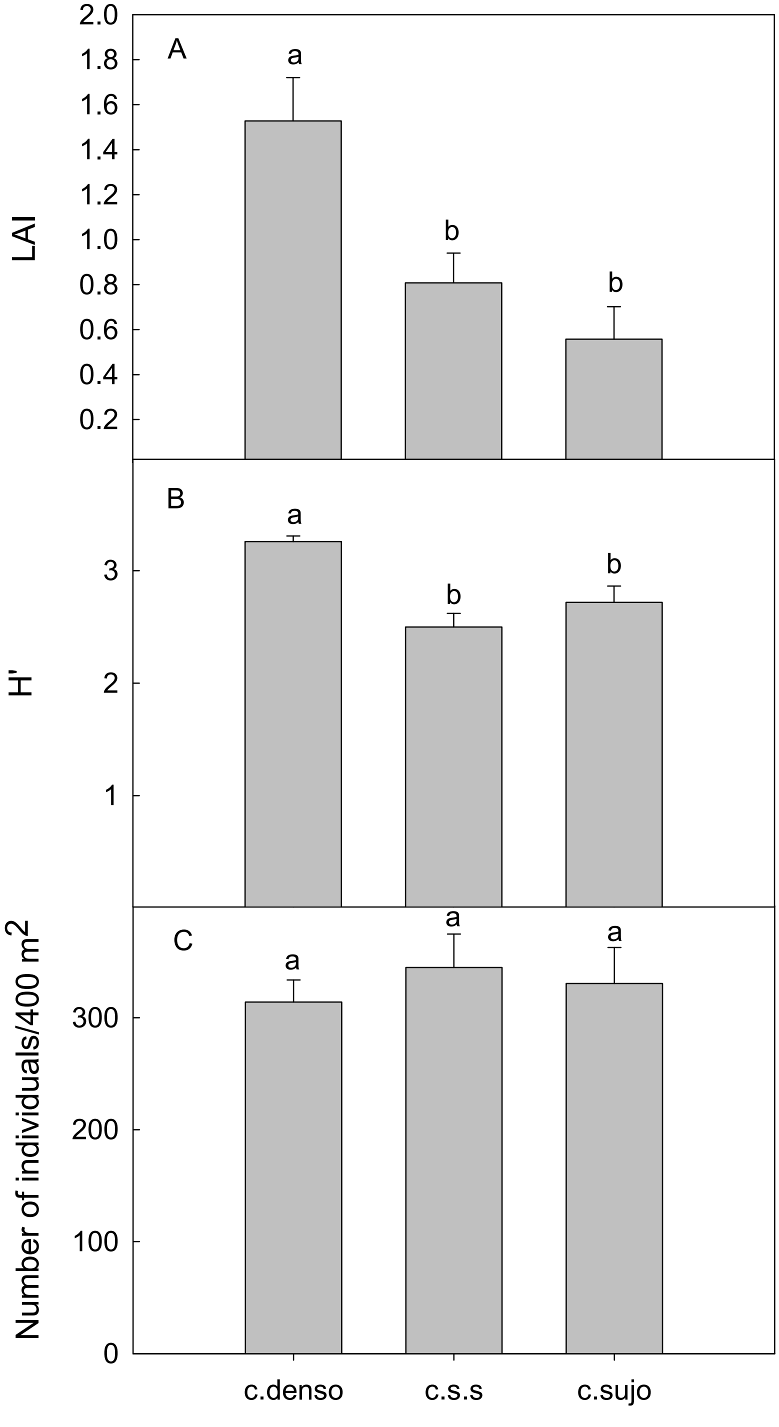


Figure 3.3 A) leaf area index (LAI), B) Shannon species diversity index (H'), and C) number of individuals of woody species taller than 1 m in each vegetation type: cerrado denso (c. denso), cerrado sensu stricto (c.s.s) and campo sujo (c. sujo). Bars are means Means + SE (n=9). Means with same letters do not differ significantly (*p* >0.05) according to Tukey HSD poshoc test and according to Kruskal-Wallis pair wise tests.

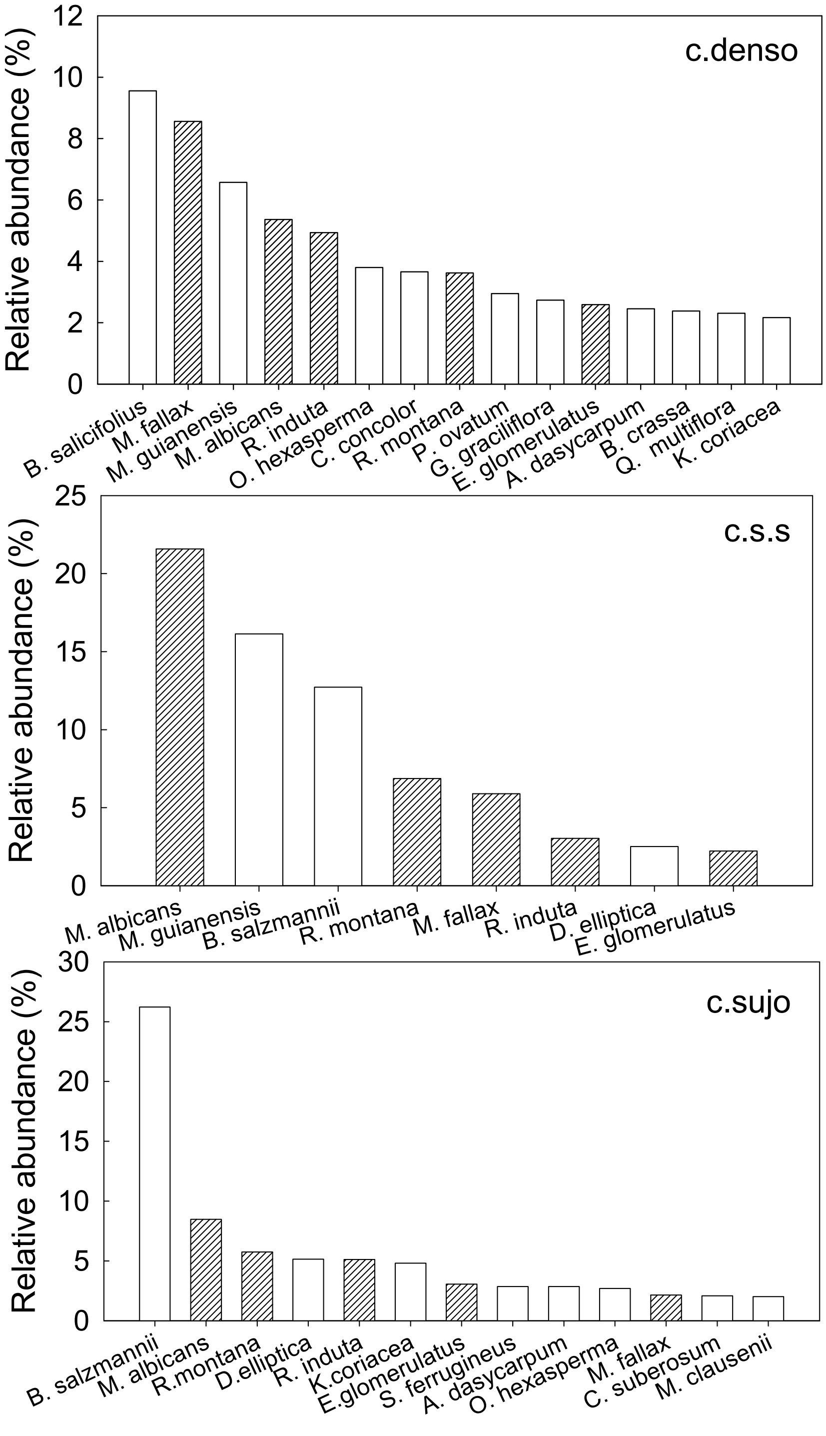
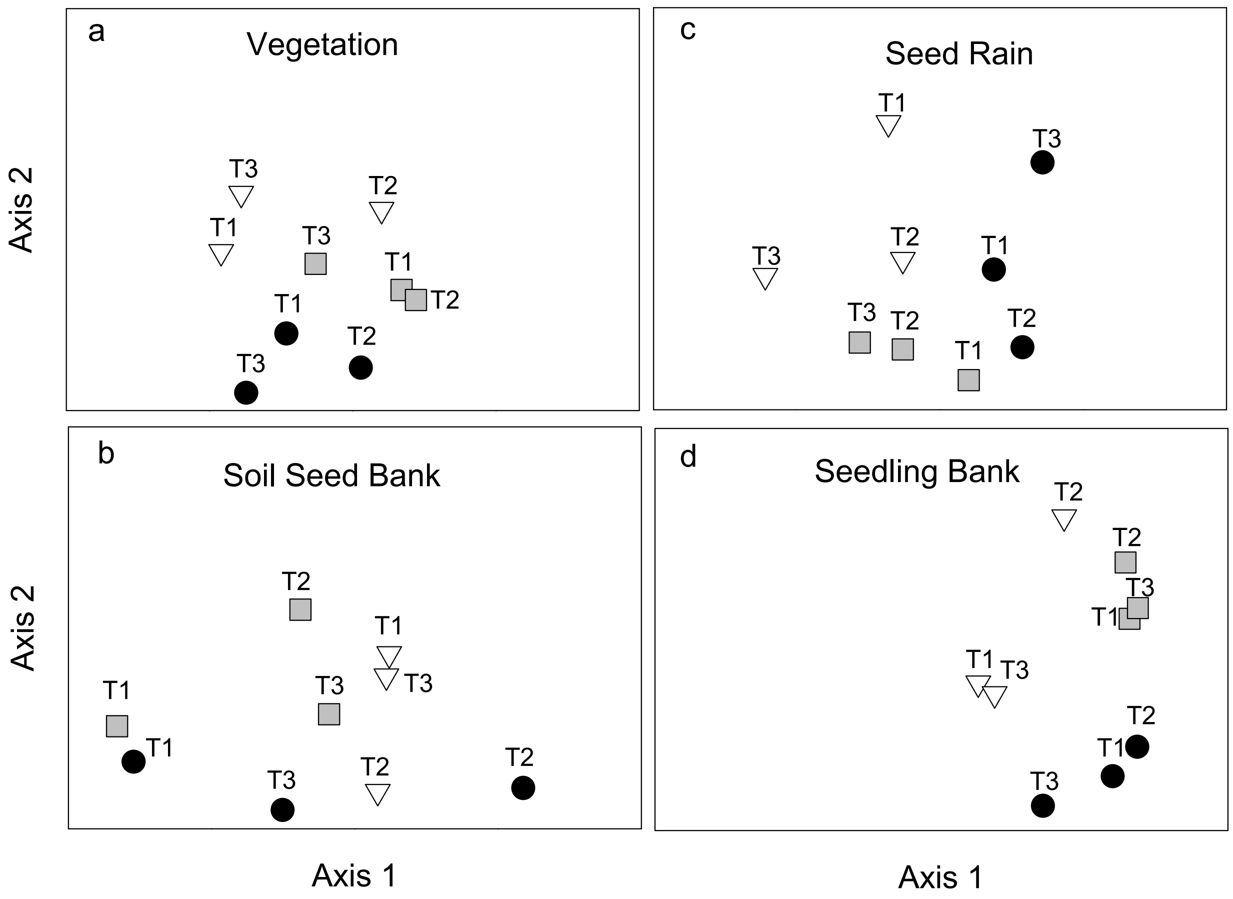


Figure 3.4. Relative abundance of individuals taller than 1 m from woody species with relative abundance > 2% in each vegetation type: cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c. sujo). Values for each species combine the abundance of the three transects. Bars with shading represent species present in all three vegetation types.

Figure 3.5. a) Non-metric multidimensional scaling (NMDS) ordination of: a) composition of woody species in each vegetation type, b) NMDS for soil seed bank of woody species, c) NMDS for seed rain of woody species, and d) NMDS for seedling bank of woody species in cerrado denso (black circles), cerrado sensu stricto (gray squares) and campo sujo (open triangles). T1, T2 and T3 correspond to Transect 1, Transect 2, and Transect 3 sites respectively. Points that are close together represent sites with similar species composition; points that are far apart correspond to sites with very different species composition.



Chapter 4

Canopy cover facilitates establishment of woody seedlings in Neotropical savannas

Seedling recruitment is a bottleneck in the population dynamics of many plant species because plants at this stage exhibit high mortality as a consequence of both environmental and competition constraints (Harper 1977). Recruitment reflects not only seed production but also the compound filtering effects of seed dispersal and seedling establishment (Uriarte et al., 2005). At local scales, successful tree establishment has to overcome two successive bottlenecks: (1) seed limitation, the absence of recruitment because of limited seed supply, and (2) establishment limitation, the absence of recruitment because of limited availability of suitable sites that provide the conditions required for a particular seed to germinate and establish (Harper 1977; Eriksson and Ehrlén 1992; Clark et al., 1998; Muller-Landau et al., 2002).

Reproduction of woody plants by seeds in the tropical savannas of central Brazil (cerrado) has long been considered a rare event (Rizzini and Heringer 1962; Sarmiento and Monasterio 1983; Sarmiento 1984). Sprouting from root suckers or rhizomes after extended drought periods or after fire events is frequently observed in trees and shrubs (Gottsberger and Silberbauer-Gottsberger 2006). Vegetative reproduction, therefore, has been thought to be the major means of reproduction in the cerrado despite most cerrado trees producing seeds, and experiments in the laboratory having proved their germination capacity (Felippe and Silva 1984; Barbosa et al., 1993; Paulillo et al., 1993; Joly 1996, Melo et al., 1998; Felfilli et al., 1999). Growing evidence has shown that seeds can germinate under natural conditions and that at least some cerrado tree seedlings develop into adult plants (Labouriau et al., 1963; Rizzini 1965; Nardoto et al., 1998; Sassaki and Felippe 1999; Franco 2002; Hoffman and Moreira 2002).

Cerrado vegetation is very rich in endemic woody species. More than 500 species of trees and large shrubs grow in the cerrado region, and individual sites may contain up to 70 or more tree species per hectare, with a similar number of shrub species (Franco 2002; Haridasan 2008). Savanna types in the cerrado differ in tree density, tree size (DBH) and canopy height. They occupy different portions along shallow altitudinal gradients (approx 30 m in elevation) from closed savannas (cerrado denso) with high tree density, and a nearly continuous canopy cover in the uppermost portions, to open savannas (campo sujo) with scattered short trees in the low portions of the topographic gradients (Eiten 2001). Intermediate savannas (cerrado sensu stricto) occupy intermediate topographic positions and exhibit intermediate canopy cover. The herbaceous-grass layer follows the opposite trend to the woody layer with high abundance in open savannas and low abundance in closed savannas (Eiten 2001). Environmental conditions in the cerrado are stressful for the survival and growth of woody seedlings because of the five month dry season (Hoffmann 2000), limited availability of soil nutrients (Haridasan 2008), and frequent burning that occurs at 1-5 years intervals, generally during the dry season (Coutinho 1990; Moreira 2000). Fire may favor species that reproduce by vegetative means, because under high fire frequency vegetative reproduction is more successful than sexual reproduction (Hoffman 1998). Conversely, increases in density of woody plants after exclusion of fire have been observed in closed and open savannas in the cerrado (Moreira 2000). In African savannas also fire protection induces gradual changes in density of tree species leading to increased density (Swaine et al., 1992) or changes in size and biomass of tree populations (Higgins et al., 2007).

Too much or too little light (Matlaga and Horvitz 2009), soil water deficits (Hoffmann 1996), nutrient availability (Barloto et al., 2006; Breen and Richards 2008), leaf litter cover (Molofsky and Augspurger 1992), and abundant grass biomass (Hagenah et al., 2009) highly constrain the quality and quantity of sites for emergence and establishment of seedlings. These factors may change among cerrado savanna types differentially affecting seedling establishment of woody species. Little is known about the establishment of woody seedlings in fire-protected savannas in the cerrado. In particular, it is not clear whether seedling recruitment and survival under natural conditions, differ among cerrado savanna types or which environmental factors limit seedling establishment the most in the absence of fire.

The general objective of this study was to evaluate the establishment of woody seedlings in three savanna types that differ in canopy cover: closed, intermediate and open savannas, in the central Brazilian plateau (cerrado) where fire has been excluded for at least 30 years. I tested the following hypotheses: (1) richness (number of species) and density (number of seedlings/m^2) of woody seedlings is higher in closed than in open savannas; (2) seedling survival and recruitment is higher in closed than in open savannas; (3) seedling limitation is lower in closed than in open savannas; and (4) litter cover and light level affect seedling emergence of woody species under controlled experimental conditions.

Methods

Study site

This study was conducted from June 2005 to July 2008 at the Instituto Brasileiro de Geografia e Estatística (IBGE) reserve, a field station located 35 Km south of Brasilia, Brazil (15˚ 56' S, 47˚ 63' W, altitude 1100 m). Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August often are rainless. Relative humidity during the rainy season is about 80% and drops to 55% during the dry season when daily minimum relative humidity may reach values as low as 10% at midday. Mean monthly temperatures ranged from 19˚C to 23˚C. The soils are very deep and well-drained oxisols. The IBGE reserve contains all the major vegetation types: cerrado denso, cerrado sensu stricto, and campo sujo. Cerrado denso is a semi-closed woodland (40%-60% tree crown cover) with a moderately tall (usually 7 m) tree canopy with a mean leaf area index (LAI) of 1.5. Because of the shade created by the canopy trees, the herbaceous and semi-woody ground vegetation exhibits less cover than in relatively open cerrado vegetation. Cerrado sensu stricto is a savanna dominated by trees and shrubs (canopy generally less than 7 m tall) with less than 40% tree crown cover and mean LAI of 0.8. Campo sujo has few short trees and scattered shrubs over a near continuous grass layer (the woody layer usually covers less than 10% of the ground) and mean LAI of 0.5.

In June 2005, I established three transects (of approximately 1000 m) in the reserve, and each spanned the three major vegetation types. Transects were placed along continuous topographic gradients with different elevations (ranging from 1117 to 1153 m a.s.l.), in vegetation types that had been protected from fire for at least 30 years. I established nine plots of 20 x 20 m along each transect (three per vegetation type), for a total of 27 permanent plots. I determined preliminary species-area curves for cerrado denso and campo sujo areas, and the results indicated that about 85% of the maximum richness was attained at a plot size of 1024 m^2. Species composition and abundance in each plot was assessed by identifying and counting all individuals of woody species taller than 1 m.

Environmental conditions at the study site

I quantified photosynthetic photon flux density (PPFD) among vegetation types by using a Decagon® AccuPAR model LP-80 PAR/LAI Ceptometer. PPFD was measured 1m above the soil surface, at midday on clear days at 30 random points in each permanent plot in the three vegetation types, during the dry season of 2007. Red/far-red wavelength ratios of transmitted light in the three vegetation types were measured at midday on clear days, during the dry season of 2006 in 9 randomly selected plots (three per vegetation type) 30 cm above the soil surface. Transmitted light (200-1100 nm) was measured with a portable USB-2000 fiber optic spectrometer (8.91 cm x 6.33 cm x 3.44 cm) connected to a portable computer. Red/far-red ratios were calculated by dividing the percent of transmitted light in the red-waveband (600-700 nm) by the percent of transmitted light in the far red wavebands (700-800 nm) using OOIBase 32^{TM} operating software (http://www.oceanoptics.com).

I also determined soil nutrient content and gravimetric water content among vegetation types by collecting soil samples at the center of each 20 x 20 m plot in July 2005 (mid dry season). I collected soil samples at 0-5cm, 5-10 cm, 10-25 cm and 25-50 cm. The superficial leaf litter was removed before soil collection. Soil sub-samples were sent to the University of Brasilia for determination of Ca (mg/Kg), N (%), P (mg/Kg) C (%), K (mg/Kg), Mg (mg/Kg), Mn (mg/Kg), Al (mg/Kg), Cu (mg/Kg), Fe (mg/Kg), Zn (mg/Kg) and pH. Soil pH was measured in 1:2.5 soil-water suspension. Exchangeable Ca, Mg and Al were determined in soil extracts of 1M KCl and exchangeable K and available P, Fe, Mn, Zn, and Cu in Mehlich's extract (a diacid mixture of 0.05M HCl and 0.0125 M H_2SO_4). The cations in soil extracts were determined by atomic absorption spectrophotometry, and P by colorimetry (Allen 1989). Total N in soil was determined by Kjeldahl digestion and distillation. Soil organic carbon was determined by the method of Walkley and Black (Allen 1989).

Extra soil sub-samples were placed in tin sample canisters (2-3/8" diameter. x 1-3/4" height) to determine gravimetric water content. In the laboratory, canisters were weighted, oven dried at 65° C for at least 72 hours until they reached a constant weight. The gravimetric soil water content was calculated as follows: ((soil fresh weight – soil dry weight)/soil dry weight) x 100%.

In July 2008 I estimated soil water potential among vegetation types using the filter-paper technique (Deka et al., 1995). Soil samples were collected at 0-5cm, 5-10 cm, 10-25 cm and 25-50 cm depth at the center of each 20 x 20 m plots. After collection, samples immediately were taken to the laboratory. Soil was placed in plastic containers (7.30 cm diameter, 6.03 cm height) in contact with two sheets of filter paper (Whatman 42; 5.5 cm diameter) placed in the middle of the container (but without touching the wall, to avoid absorption of moisture that may condense on the wall). The containers were sealed with masking tape to minimize water loss and immediately were placed in a thermally insulated box to avoid thermal distillation once the temperature in the box had stabilized. The samples were allowed to equilibrate for ten days in the laboratory. After equilibration, the soil in each container was emptied onto a dish and the filter paper was removed with tweezers. Soil adhering to the paper was lightly brushed off and the papers were weighed immediately. Filter papers were oven dried at 65 ˚for at least 72 hours until they reached a constant weight, and then were reweighed to measure their water content. The change in mass of each filter paper was related to the soil water potential through a previously determined calibration equation for Whatman filter paper no 42: $\log_{10}(-\psi m) = 5.144 - 6.699\, w$, where ψm is the water potential and w is the gravimetric water content ($g\ g^{-1}$).

Seedling density and survival

To quantify seedling richness, density and survival in June 2005 I established eight sub-plots of 1 x 1 m within each of the 20 x 20 m plots for a total of 216 plots (72 per vegetation type). I performed preliminary species-plot curves in cerrado denso and campo sujo areas that indicated that about 90% of the maximum richness was attained using eight 1 x 1m plots. Seedling plots were established randomly within each 20 x 20 m plot. In July 2006, all seedlings up to 30 cm tall in the plots were tagged with a unique number, and identified to morpho-species. Subsequent censuses took place in March 2007, July 2007, November 2007 and July 2008. During each census, newly recruited seedlings were tagged, and missing seedlings were recorded as “dead”. Seedlings were identified at the species level (or family when it was not possible to identify the species) by comparing seedling specimens growing in the vicinity of the subplots with reference specimens at the IBGE herbarium, help of experts, and published literature. The initial seedling assemblage included seedlings established before July 2006; seedlings recruited from July 2006 to March 2007 made up Cohort 1; seedlings recruited from March 2007 to July 2007 made up Cohort 2; and seedlings recruited from July 2007 to November 2007 made up Cohort 3.

Seedling limitation

I quantified specific seedling limitation (Clark et al., 1998 and Muller-Landau et al., 2002) of common woody species found in the initial seedling assemblage and in each cohort, in all vegetation types. Seedling limitation was calculated as the proportion of subplots where seedlings of a particular woody species did not occur. (Seedling Limitation = 1- (r/*n*) where *r* is the number of subplots where seedlings of a particular woody species occur, and *n* is the number of subplots (i.e. 8 subplots in each 20 x 20 m permanent plot). I also quantified community wide seedling limitation in the initial seedling assemblage and in each cohort as the proportion of subplots in each 20 x 20 m plot without seedlings of any woody species.

Effect of litter cover on seedling emergence

To quantify litter-cover among vegetation types, I took litter samples from each permanent plot at three random points, during March 2006. All litter contained in squares of 25 x 25 x 5cm was collected and oven dried at 65°C until it reached a constant weight. Mature seeds from several individuals of nine woody species were collected in the field and mixed to form a bulked sample. These species (*Acosmium dasycarpum* (Fabaceae), *Brosimum gaudichaudii* (Moraceae), *Eriotheca pubescences* (Malvaceae), *Guapira graciliflora* (Nyctaginaceae), *Guapira noxia* (Nyctaginaceae), *Miconia ferruginata* (Melastomataceae), *Ouratea hexasperma* (Ochnaceae), *Palicourea rigida* (Rubiaceae), and *Solanum lycocarpum* (Solanaceae)) are common and widely distributed throughout much of the cerrado (Silva-Junior 2005). To examine the effect of litter cover on seedling emergence we used an experimental design with three levels of litter cover and five replicates of 20 seeds each under greenhouse conditions. Litter was collected from random plots of each vegetation type. A bulked sample of oven-dried mixed litter from different species was used to cover the trays. Seeds were sown in plastic trays (25 cm x 30 cm x 4cm) filled with an artificial substrate (mix of 70% peat moss and 30% vermiculite) for a total of 15 trays per species. Five trays were covered with 25g of dry litter (2g/cm^2), five trays were covered with 50g of dry litter (4g/cm^2), and five control trays received no litter. The trays were randomly distributed on the greenhouse benches, under temperature conditions of 25-28ºC, 32% full sunlight, and watered daily with an automated sprinkler system. Seedling emergence, defined as emergence of cotyledons, was monitored every three days up to 4-5 weeks (depending on the species).

Effect of light level on seedling emergence

To examine the effect of light level on seedling emergence of cerrado woody species I used a factorial experimental design with four light levels and five replicates of 20 seeds each under greenhouse conditions. The same species mentioned above were used. Seeds were collected in the field and mixed to form a bulked sample. Seeds were sown in plastic trays (25 cm x 30 cm x 4cm) filled with an artificial substrate (mix of 70% peat moss and 30% vermiculite) for a total of 20 trays per species. Five trays were covered with shade-cloths to provide 16% ambient light (262µmol/m^2/s), five trays were covered with shade-cloth to provide 20% ambient light (350µmol/m^2/s), five trays were covered with aluminum foil to produce 0% ambient light (0.5µmol/m^2/s), and five control trays were not covered and received 32% full sunlight (525µmol/m^2/s). To allow water and oxygen diffusion several holes (1mm) were opened in the aluminum foil. Trays were randomly distributed on greenhouse benches, under temperature conditions of 25-28ºC, and were watered daily with an automated sprinkler system to field capacity. Seedling emergence, defined as emergence of cotyledons, was monitored every three days for 4-5 weeks (depending on the species).

Data analysis

The effect of vegetation type and time on seedling density of each cohort was analyzed with a mixed model approach (Pinheiro and Bates 2000) with vegetation as the fixed factor and time as a random factor. Transect also was included as random factor. Because the response variable (seedling density) departed from a normal distribution, a Poisson error distribution structure was used. To estimate the model parameters I used a generalized linear mixed model (GLMM). Minimum adequate models were obtained by deleting non-significant terms ($p > 0.05$) from the full model consisting of all variables and their interactions. The model selection procedure was based on a penalized likelihood measure of the goodness of fit, the Akaike Information Criterion (AIC). The effect of vegetation type (fixed effect) and time (random effect) on richness and density of recruited seedlings also was analyzed with GLMM using a Poisson error distribution. The effect of vegetation type (fixed effect) and time (random effect) on the ratio richness/density of recruited seedlings also was analyzed with GLMM using a normal error distribution structure. These statistical analyzes were performed in R 2.91 (http://www.R-project.org). I used the package "lme4" for generalized linear mixed models.

I used Kaplan-Meier survival analysis (Fox 2001) to analyze the effects of vegetation type on survival of seedlings I estimated cohort survivorship ('survival function') and the probability of surviving the first eight months and of surviving to the end of the study period for each cohort within each vegetation type. Pair-wise comparisons of the survival functions of seedlings among vegetation types were performed with a Log-Rank test (Krebs1999).

Differences in seedling limitation of woody species among vegetation types in each cohort were examined using non-parametric Kruskal-Wallis tests. The data from the three transects of each vegetation type were pooled. Differences in leaf area index, PFFD, red:far-red ratios, and soil nutrient concentrations among vegetation types were examined with one-way ANOVAs. Tukey HSD post hoc tests were used for multiple comparisons of means. Differences in soil gravimetric water content and soil water potential among vegetation types and collection depth were examined with 2-way factorial ANOVAs with vegetation type and soil depth as main fixed factors. Differences in seedling emergence under different litter cover and under different light levels among vegetation types and species were examined with 2-way factorial ANOVAs with vegetation type and species as main fixed factors. For each species, separate one-way ANOVA tests were performed to examine the effect of litter cover and light level on seedling emergence. Tukey HSD post hoc tests were used for multiple comparisons of means. These analyses were performed with JMP 7 (SAS Cary, NC).

Results

Environmental conditions at the study site

Photosynthetic photon flux density (PPFD) was significantly lower in cerrado denso (400.97±37.14) than in cerrado sensu stricto (1366.00± 36.80) and campo sujo respectively (1558.96±2.62), and the latter two also differed significantly (One-way ANOVA, $F_{(2, 272)} = 402.92$, $p<0.0001$; Figure 4.1A). Consistent with differences in tree canopy cover, red: far-red ratios of transmitted light also differed significantly among the vegetation types (One-way ANOVA, $F_{(2, 65)} = 35.13$, $p<0.0001$; Figure 4.1B) with lower red: far-red ratio in cerrado denso (0.42±0.01) than in cerrado sensu stricto (0.50 ± 0.01) and campo sujo (0.57±0.01). Significant differences in soil nutrient concentrations among vegetation types were found for calcium, potassium aluminum, manganese and copper (Table 4.1). Nitrogen, phosphorus and carbon did not differ among vegetation types; pH was significantly lower in soils from cerrado denso than in soils from cerrado sensu stricto and campo sujo (Table 4.1). Soil water potential differed significantly among soil depths, but not among vegetation types (2-way ANOVA, $F_{\text{Veg}\,(2,96)} = 1.03$; $p = 0.36$; $F_{\text{Depth}\,(3, 96)} = 41.18$; $p<0.0001$; $F_{\text{Veg x Depth}\,(6,96)} = 0.15$; $p = 0.98$). Soil water potential, as expected, was significantly higher (more negative) in the upper parts of the soil profile (5 cm-10 cm) than in deeper portions of the soil profile (25 and 50 cm)

(Figure 4.2A). Likewise, depth, but not vegetation type had a significant effect on gravimetric soil water content (2-way ANOVA, $F_{\text{Veg}\ (2,96)} = 1.50$; $p = 0.23$; $F_{\text{Depth}\ (3,96)} = 211.91$; $p < 0.0001$; $F_{\text{Veg x Depth}\ (6,96)} = 1.14$; $p = 0.35$). Mean gravimetric water content significantly decreased in the upper portion of the soil profile from 37.87% at 50 cm to 18.66% at 5 cm (Figure 4.2B).

Seedling density and recruitment

During the study period I censused a total of 2815 seedlings (1917 woody, 388 herbaceous, and 510 undetermined seedlings). I marked 1347 seedlings (950 woody, 180 herbaceous, and 217 undetermined seedlings) during the initial census (initial seedling assemblage), and a total of 1468 recruits (967 woody, 208 herbaceous, and 293 undetermined seedlings) in the three subsequent censuses which constituted the three cohorts studied. The total number of woody seedlings in the three cohorts was higher than the number of herbaceous seedlings in all three vegetation types. Therefore, the following results refer only to seedlings of woody species.

Density (number of seedlings per m^2) of the initial seedling assemblage was higher than the densities of successive cohorts (Figure 4.3). Mean density of the initial seedling assemblage was 3.38, mean density of Cohort 1 recruits was 2.17, mean density of Cohort 2 recruits was 0.81, and mean density of Cohort 3 recruits was 0.72. Density of the initial seedling assemblage, and the three cohorts studied decreased over time in all three vegetation types (Figure 4.3). The effect of time on seedling density was marginally significant ($p = 0.06$) only in Cohort 3 (Table 4.2). Seedling density differed significantly ($p < 0.05$) between cerrado denso and cerrado sensu stricto, and between cerrado denso and campo sujo in these three cohorts but not in the initial seedling assemblage (Table 4.2). In a separate analysis I found that the density of Cohort 1 recruits differed significantly ($p< 0.05$) between cerrado sensu stricto and campo sujo. However, the densities of Cohort 2 and 3 recruits did not differ significantly ($p> 0.05$) between cerrado sensu stricto and campo sujo.

Richness (number of species /m^2) of recruits also decreased over time, in particular between the first and second censuses (Figure 4.4a, Table 4.3). Mean richness across vegetation types decreased over time from 2.23 in the first cohort to 0.73 in the third cohort, while mean density decreased from 2.93 in the first cohort to 0.81 in the third cohort. Richness of recruits did not differ between cerrado denso and cerrado sensu stricto, nor between cerrado denso and campo sujo (Table 4.3) but it was higher in cerrado sensu stricto than in campo sujo (p=0.007). However, when I removed the effect of density on richness by taking the ratio of richness/density I found significant differences between cerrado denso and cerrado sensu stricto (p= 0.0006) and between cerrado denso and campo sujo (p=0.002) but not between cerrado sensu stricto and campo sujo (p=0.70) (Table 4.3).

Although density of recruits did not differ between cerrado denso and cerrado sensu stricto (Table 4.3), density was significantly higher in cerrado denso than in campo sujo (Table 4.3). Density of recruits also was higher in cerrado sensu stricto than in campo sujo (p<0.001). A significant interaction between vegetation type and time was found for richness and density of recruits (Table 4.3) indicating that differences in diversity of recruits among vegetation types could change through time.

Seedling survival

After 8 months, survival of initial seedlings was significantly higher in campo sujo than in cerrado denso and cerrado sensu stricto (Figure 4.5A; log-rank test of homogeneity of survival c.denso vs. c.s.s: χ^2= 0.42, df = 1, p=0.52; log-rank test of homogeneity of survival c.denso vs. c.sujo: χ^2= 6.03, df = 1, p <0.01; c.s.s vs. c.sujo: χ^2= 10.24, df = 1, p =0.001). After 24 months, however, survival of the initial seedling assemblage did not differ among the vegetation types (Figure 4.5A; log-rank test of homogeneity of survival: χ^2= 1.81, df = 2, p = 0.40). In contrast, after 8 months survival of seedlings in Cohort 1 was significantly higher in cerrado denso and cerrado sensu stricto than in campo sujo (Figure 4.5B; log-rank test of homogeneity of survival c.denso vs. c.s.s: χ^2= 1.09, df = 1, p=0.30; log-rank test of homogeneity of survival c.denso vs. c.sujo: χ^2= 20.32, df = 1, p <0.0001; c.s.s vs. c.sujo: χ^2= 12.88, df = 1, p =0.0003). The same pattern of survival was observed after 16 months (Figure 4.5B, log-rank test of homogeneity of survival c.denso vs. c.s.s: χ^2= 0.15, df = 1, p=0.70; log-rank test of homogeneity of survival c.denso vs. c.sujo: χ^2= 22.50, df = 1, p <0.0001; c.s.s vs. c.sujo: χ^2= 24.87, df = 1, p <0.0001). Although not significant, seedling survival of Cohort 2 was consistently higher in cerrado denso than in campo sujo after 8 and 12 months (Figure 4.5C; log-rank test of homogeneity of survival Cohort 2: χ^2= 1.81, df = 2, p = 0.40; log-rank test of homogeneity of survival Cohort 3: χ^2= 1.95, df = 2, p = 0.37). The same pattern was observed in seedling survival of Cohort 3 after 8 months (Figure 4.5D.)

Seedling limitation

Community seedling limitation, measured as the proportion of 1m^2 subplots without woody seedlings, differed across cohorts (Figure 4.6; H = 43.84, df = 3, p <0.001). The mean proportion of sites not occupied by woody seedlings increased with subsequent cohorts from 0.07 in initial seedlings, to 0.12 in Cohort 2, 0.35 in Cohort 3 and 0.48 in Cohort 4. Although the proportion of sites without seedlings did not differ significantly among vegetation types within each cohort ($p>0.05$), sites in cerrado denso consistently had more seedlings than sites in cerrado sensu stricto and in campo sujo, respectively (Figure 4.6). The number of new recruits in each plot significantly decreased as the number of pre-existing seedlings increased in cerrado denso but not in cerrado sensu stricto or in campo sujo (Figure 4.7).

In all three vegetation types, seedling limitation of 16 woody species selected for individual study was relatively high and differed among cohorts (Table 4.4). Significant differences among the three vegetation types ($p<0.05$) were found only for five species of Cohort 1 (*Campomanesia pubescens*, *Miconia albicans*, *Miconia fallax*, *Myrsine guianensis*, and *Rourea induta*). Mean seedling limitation, however did not differ significantly among the vegetation types in Cohort 1 (Table 4.4). In Cohort 2, seedling limitation of six species (*Dalbergia miscolobium, Eugenia bracteata*, *Myrsine guianensis*, *Ouratea floribunda, Roupala Montana*, and *Rourea induta*) differed significantly among the vegetation types. Mean seedling limitation in this cohort was significantly higher in campo sujo than in cerrado sensu stricto and cerrado denso (Table 4.4). In Cohort 3, seedling limitation of four species (*Casearia altiplanensis* , *Dalbergia miscolobium* , *Miconia albicans* ,and *Ouratea floribunda*) differed significantly among the vegetation types. Mean seedling limitation in this cohort was also significantly higher in campo sujo and cerrado sensu stricto than in cerrado denso (Table 4.4).

In general, mean seedling limitation of Cohorts 2 and 3 (new recruits) was higher than that of Cohort 1.

Seedling emergence under different litter cover

In the field, litter cover was significantly higher in cerrado denso than in cerrado sensu stricto and campo sujo (Figure 4.8, insert); however, litter cover did not differ between cerrado sensu stricto and campo sujo. In the greenhouse experiment, seedling emergence differed widely among species ranging from 5.33% in *G. graciliflora* to 74% in *B. gaudichaudii* (Figure 4.8). Litter cover, species, and their interaction had a significant effect on seedling emergence (2 way-ANOVA, $F_{\text{litter }(2, 103)} = 7.98$, $p=0.0006$; $F_{\text{species }(8, 103)} = 40.81$, $p < 0.0001$; $F_{\text{litter x species }(16, 103)} = 2.63$, $p= 0.002$) indicating that the effect of litter cover depended on the species. Mean seedling emergence of species with no litter cover (33.11% ± 4.16) was higher than mean seedling emergence with 4g/cm^2 litter cover (22.26% ± 3.01). Mean seedling emergence of species with 2 g/cm^2 (27.16 %± 3.76) , and with 4g/cm^2 litter cover did not differ significantly; nor mean emergence of species with no litter and 2 g/cm^2 litter. Seedling emergence of *B.gaudichaudii*, *P.rigida*, *G.noxia*, and *A.dasycarpum* were significantly affected by litter cover. For these species, emergence of seedlings with high litter cover (4g/cm^2) was significantly lower than emergence of seedlings with no litter cover or low litter cover 2g/cm^2 (Figure 4.8).

Seedling emergence under different light levels

In the greenhouse, emergence of seedlings across light levels differed among species ranging from 3% in *E. pubescens* to 68% in *B. gaudichaudii*. Light level, species, and their interaction had a significant effect on seedling emergence (2 way-ANOVA, $F_{\text{ligh (3,132)t}} = 20.35$, $p<0.0001$; $F_{\text{species (8,132)}} = 31.98$, $p < 0.0001$; $F_{\text{light x species (24, 132)}} = 4.82$, $p= 0.002$) indicating that the effect of light level depended on the species. On average, seedling emergence of species with 0% ambient light was significantly lower (9.29% ± 2.49) than seedling emergence of species 32% ambient light (control: 33.57 ± 4.62), 20% ambient light (30.24% ±4.77), and 16% ambient light (24.28% ± 4.54). Emergence of seedlings with 32% ambient light (control) and seedlings with 16% ambient light differed significantly. However, emergence of seedlings with 32% ambient light and seedling with 20% ambient light did not differ, nor did emergence of seedlings with 20% and 16% ambient light. Seedling emergence of *B. gaudichaudii, P.rigida, M.ferrugianta, G. graciliflora,* and *G. noxia* were significantly affected by light level (Figure 4.9). For those species, emergence of seedlings with 0% ambient light (100% shade) was significantly lower than emergence of seedlings with no shade or low shade levels, suggesting a negative effect of shade on seedling emergence.

Discussion

Seedling establishment

Density of all three cohorts of recruits was significantly higher in cerrado denso than in campo sujo suggesting that seedling establishment is higher in closed savannas than in open savannas. These differences in density could be the consequence of a positive (nurse) effect of canopy cover on seedling establishment. Our results agree with other studies that also found higher establishment of tropical savanna trees under high canopy cover than in open grassland (Hoffman 1996; Sassaki and Felipe 1999; but see Kanegae et al., 2000). Canopy cover might help to ameliorate soil water stress by reducing temperature fluctuations at the soil surface during germination or shortly thereafter when seedlings are most sensitive to drought (Hoffman 1996). Partial shading by tall woody plants, particularly in cerrado denso could also facilitate establishment of woody seedlings by reducing stressful heat conditions of campo sujo areas (Gottsberger and Silberbauer -Gottsberger 2006). The higher density of woody seedlings in cerrado denso and cerrado sensu stricto than in campo sujo also could be facilitated by the lower competition with herbaceous species in closed and intermediate savannas than in the open savannas, where herbaceous plant densities are substantially high. Studies have demonstrated that removal or clipping of exotic and native grasses can increase establishment, survival and growth of savanna tree seedlings (Williams et al., 2005; Hagenah et al., 2009). Hoffmann and Haridasan (2008) found that survival of cerrado tree seedlings was negatively correlated with grass biomass.

I found that seedling density significantly decreased over time in all woody species cohorts in all three vegetation types, and this reduction was the result of mortality during both dry and wet seasons. Mean seedling survival across cohorts, however, was higher than 50%, despite some seedlings having experienced two consecutive dry seasons. In fire protected sites, Hoffmann (1996) found high survival rates of cerrado woody species despite a prolonged dry season. Kanegae et al (2000) reported between 67% and 85% survival in 1 year old seedlings of *Bowdichia virgiloides* in campo sujo areas. Survival of *Kielmeyera coriaceae* seedlings was 64% in a cerrado near Brasilia five years after emergence (Oliveira and Silva 1993). In our study, the high survival of woody seedlings in all cohorts (>60%), particularly during the first 8 months, could be the result of fast root development and swollen root systems during early seedling establishment. Many savanna species typically have deep taproots that develop quickly in the seedling stage (Oliveria and Silva 1993). These taproots play an important role in drought tolerance of seedlings (Hoffmann 2000) by allowing access to deep soil water during the dry season (Hoffmann and Franco 2003). Recurrent sprouting of seedlings from early developed swollen root systems also has been shown to ensure survival in several cerrado woody species (Oliveria and Silva 1993; Moreira and Klink 2000). In our study I found that soil gravimetric water content at 50 cm depth was about 40% during the dry season of 2005, and soil water potential at 50 cm depth did not drop below -0.5 Mpa in any vegetation type during the dry season of 2008 (Figure 4.6).These results suggest that there is available water at 50 cm depth which could be reached by deep seedling roots. In our study, seedling survival was consistently lower in open than in closed savannas in all cohorts, with significant differences between cerrado denso and campo sujo only in seedlings of Cohort 1. In a separate survival analysis, however, I observed that survival rates in all cohorts differed significantly among species ($p<0.001$). This suggests that the probability of survival of woody seedling also depends on the species. In this study, survival of seedlings decreased through time. Average survival of initial seedlings across vegetation types decreased from 84.33% to 50% within 16 months. Average survival of Cohort 1 recruits decreased from 68% to 61% within 8 months; and survival of Cohort 2 recruits decreased from 79.66% to 71% in 4 months. These results indicate that older recruits were more susceptible to mortality events than new recruits. One possible explanation for this age-related mortality could be high spatial competition in cohorts with high seedling density. Because density of older recruits was higher than density of new recruits; high seedling densities could have increased mortality. Survival of new recruits could decrease in following seasons once they experience further abiotic and biotic constraints. For example, rain-free periods during the wet season are frequent in the cerrado and are known to desiccate young seedlings (Franco 2002). Differential survival between old and new seedlings also could reflect differential herbivore pressure. Tropical seedlings in Panama were more attacked by insect herbivores and vertebrate browsers during a late developmental stage when they already had branched repeatedly (Dalling and Hubbell 2002). Nardoto et al., (1998) found that armadillos enhanced mortality of *Kielmeyera coriaceae* seedlings by 35% in campo sujo during the second year of being transplanted to the field. Although the impact of herbivory on cerrado seedling dynamics is unknown, the diverse and abundant communities of herbivores, in particular insects, might affect seedling survival. For example, plant consumption by leaf cutter ants (*Atta* spp) in the cerrado has been found to be high (Costa et al., 2008) and could drive shifts in plant community composition (Rao et al., 2001).

In this study, recruitment decreased significantly over time. The proportion of sites occupied by seedlings across vegetation types decreased from 0.88 in Cohort 1 to 0.52 in Cohort 3 (Figure 4.4). The low abundance of new recruits, particularly in the open savannas could be the result of a decrease in available micro-sites over time. I found that the number of new recruits in each plot significantly decreased as the number of pre-existing seedlings increased in cerrado denso but not in cerrado sensu stricto or campo sujo. In a separate analysis I found that the number of new recruits in each transect significantly decreased as the number of pre-existing seedlings increased in cerrado denso and cerrado sensu stricto but not in campo sujo (results not shown). These results suggest that other factors than density might constrain recruitment in open savannas. Because recruitment limitation reflects a combination of seed limitation and establishment limitation (Muller-Landau et al., 2002), recruitment of woody species in our study could be limited by both seed supply and seedling establishment. In a parallel study I found strong seed limitation for several woody species in all three vegetation types, with higher seed limitation in campo sujo than in cerrado denso. Seed limitation was the result of poorly dispersed seeds among sites, rapid loss of seed viability, and high rates of seed removal and predation. The results of the present study suggest that in addition to seed limitation, seedling establishment limitation constrains recruitment of woody species in the cerrado. In fact, I found high specific seedling limitation for most of the 16 woody species in all cohorts in each savanna types and mean limitation values were higher in open savannas than in closed savannas. Nevertheless, seedlings of *Campomanesia pubescens*, and *Myrsine guianensis* in Cohort 1, and *Eugenia bracteata* in Cohort 2 exhibited high limitation in cerrado denso, suggesting that seedlings of some species can establish better in open than in closed savannas. Therefore, seedling establishment also depends on particular species-specific site requirements (see below).

Environmental effects on seedling emergence

The differences in irradiance, light quality, and litter cover among savanna types indicate substantial environmental heterogeneity in the cerrado landscape from open to closed savannas. Such heterogeneity in the environment could result in differential availability of optimal microsites for seedling establishment from cerrado denso to campo sujo. In the greenhouse experiment, light level and litter cover affected seedling emergence of woody species, but their effects were species-specific. These results suggest that requirements for germination and early seedling establishment differ greatly among cerrado woody species.

In the greenhouse experiment, most species had low emergence responses to large quantities of litter cover (Figure 4.8). Studies have shown that litter cover negatively affects germination and seedling emergence of several species. Density and diversity of seedlings of deciduous species responded positively to litter removal treatments (Baeten et al., 2009); seedling emergence of pioneer species in central Panama was almost three-fold higher in litter free plots than in plots with litter (Dalling and Hubbell 2002); and germination and seedling emergence of relatively small-seeded tropical species also has been found strongly inhibited by surface litter (Molofsky and Augspurger 1992; Vázquez-Yanes and Orozco-Segovia 1993). Litter can be a physical barrier for the emergence of seedlings; in particular those derived from small seeds that lack the reserves required to grow sufficiently fast to penetrate the litter or reach the soil through the litter layer (Vázquez-Yanes and Orozco-Segovia 1993). In our study, most species had small seeds, so it is likely that litter was a physical barrier to seedling emergence, in agreement with the results of the litter cover experiments. In addition to litter acting as a physical barrier, litter cover could have reduced seedling emergence by modifying other micro-environmental conditions such as local humidity. I observed higher fungus proliferation in litter cover trays than in litter-free trays. Studies also have shown that high humidity increases pathogen proliferation and releases toxic allelopathic substances that negatively affect seedlings (Scarpa and Valio 2008; Hovstad and Ohlson 2008). In the field, however, rapid litter decomposition at the beginning of the wet season could reduce substantially litter cover thickness on the soil surface (Villalobos, unpublished results) thus favoring establishment of small-seeded species.

In our experiment, seedling emergence of several species was strongly inhibited under 0% full-sun light (100% shade) (except for *A.dasycarpum*, *E.pubescens*, *O. hexasperma*, and *S. lycocarpum*), suggesting that some species are able to detect differences between dark versus 20% or 16% of full sun light microenvironment and remain dormant in darkness. In cerrado denso, PPFD ranged from 37 to 1363 µmol/m2/s with a mean of 400.97µmol/m2/s, probably not low enough to reach darkness and thus inhibit seedling emergence of most species. Results from this experiment do not allow us to speculate about the response of seedling emergence and survival to light quality under natural conditions because neutral density filters were used to alter light levels reaching the seeds. However, the spectral composition of the light transmitted at 30 cm above the soil differed significantly among vegetation types, with light enriched in far red in closed savannas versus open savannas. Shade caused by grasses, particularly in cerrado sensu stricto and campo sujo, also could contribute to spectral differences in the light environment that seeds may perceive. Germination and seedling growth of species with photosensitive seeds are particularly affected by changes in light spectra (Vázquez - Yanes et al., 1990;Valio and Scarpa 2001) because seeds may differ in their sensitivity to a particular ratio of red/far-red transmitted light (Vázquez-Yanes et al., 1990; Batalla et al., 2000).

Overall, our results indicate that richness and density of woody seedlings was higher in closed (cerrado denso) than in open savannas (campo sujo). The difference in density between savanna types was the result of high survival and recruitment under relatively high canopy cover. Canopy cover seemed to facilitate establishment of seedlings by buffering seedlings against high irradiance and water deficit, particularly during dry periods. Consequently, species-specific seedling limitation of woody species was consistently higher in open savannas than in closed savannas. Seedling establishment and dynamics of woody species were consistent with the pattern of canopy cover variation across the cerrado landscape. Litter cover, light quality, and light availability differed significantly from cerrado denso to campo sujo. Species responded differently to those environments under experimental conditions. High litter cover and shade significantly reduced seedling emergence of some, but not all woody species. These results suggest that heterogeneity in both space and time contribute to the differential establishment of seedlings among savanna types of central Brazil.

Table 4.1. Soil nutrient concentrations and pH in cerrado denso (c.denso), cerrado sensu stricto (c.s.s.) and campo sujo (c. sujo). Values are means ± SE (n=9) of samples taken at 0-5, 5-10, 10-25 and 25-50 cm soil depth during the dry season of July 2005. Bold rows indicate nutrients that differ significantly (*p*< 0.05) among vegetation types according to Tukey HSD post hoc test. For those nutrients, means with the same letter do not differ significantly among vegetation types.

	Depth (cm)	**c.denso**	**c.s.s**	**c.sujo**
Ca (mg/Kg)	5	14.53 ± 1.73	11.65 ± 0.69	16.00 ± 1.46
	10	9.79 ± 0.93	6.98 ± 0.66	9.11 ± 0.67
	25	**8.05 ± 0.49a**	**5.98 ± 0.35b**	**7.02 ± 0.63ab**
	50	6.76± 0.57	4.97 ± 0.64	6.28 ± 0.94
	Mean	**9.66 ± 0.62 a**	**7.39 ± 0.42 b**	**9.61 ± 0.70 a**
N (%)	5	0.18 ± 0.008	0.16 ± 0.01	0.17 ± 0.008
	10	0.14 ± 0.004	0.13 ± 0.006	0.13 ± 0.007
	25	0.11 ± 0.005	0.10 ± 0.006	0.10 ± 0.009
	50	**0.07± 0.002b**	**0.07 ± 0.002b**	**0.11 ± 0.01a**
	Mean	0.12 ± 0.01	0.12 ± 0.02	0.13 ± 0.01
P (mg/Kg)	5	0.77 ± 0.08	0.69 ± 0.01	0.61 ± 0.14
	10	0.54 ± 0.10	0.31 ± 0.12	0.23 ± 0.12
	25	0.08 ± 0.08	0.08 ± 0.08	0 ± 0
	50	0 ± 0	0 ± 0	0 ± 0
	Mean	0.34 ± 0.04	0.26 ± 0.04	0.21 ± 0.06
C (%)	5	2.24 ± 0.15	2.23 ± 0.19	2.36 ± 0.04
	10	1.71 ± 0.07	1.72 ± 0.12	1.91 ± 0.02
	25	**1.31 ± 0.05b**	**1.33 ± 0.07b**	**1.55 ± 0.05a**
	50	0.87 ± 0.06	0.93 ± 0.10	0.95± 0.04
	Mean	1.53 ± 0.07	1.53 ± 0.08	1.69 ± 0.02
K (mg/Kg)	5	48.16 ± 3.21	50.66 ± 1.99	56.60 ± 2.94
	10	33.99 ± 2.20	31.99 ± 2.17	37.19 ± 1.25
	25	24.48 ± 1.65	20.00 ± 1.08	24.10 ± 1.45
	50	9.67± 1.20	7.91 ± 0.56	9.98 ± 0.93
	Mean	**29.08 ± 1.61ab**	**28.83 ± 1.11b**	**31.97 ± 1.35a**
Mg (mg/Kg)	5	43.13 ± 1.38	39.71 ± 1.25	45.59 ± 2.40
	10	36.87 ± 1.40	36.62 ± 1.16	39.72 ± 2.62
	25	34.21 ± 1.65	32.89 ± 0.87	34.98 ± 2.41
	50	32.08± 1.55	28.33± 1.22	29.84± 2.94
	Mean	36.57 ± 1.27	33.82 ± 0.84	37.53 ± 2.43
Mn (mg/Kg)	5	**5.78 ± 0.74b**	**6.86 ± 0.97b**	**10.80 ± 0.48a**
	10	**3.90 ± 0.43b**	**3.98 ± 0.45b**	**6.19 ± 0.57a**
	25	2.70 ± 0.51	2.95 ± 0.41	4.02 ± 0.55
	50	2.00 ± 0.40	2.26 ± 0.35	2.80 ± 0.44
	Mean	**3.60 ± 0.39 b**	**3.91 ± 0.24 b**	**5.95 ± 0.44 a**
Al (mg/Kg)	5	1.11 ± 0.09	0.91 ± 0.12	0.81 ± 0.05
	10	0.64 ± **0.05a**	0.48 ± **0.06ab**	0.41 ± **0.04b**

	25	**0.33 ± 0.02a**	**0.23 ± 0.02b**	**0.22 ± 0.02b**
	50	**0.11 ± 0.01a**	**0.08 ± 0.008ab**	**0.05 ± 0.005b**
	Mean	**0.55 ± 0.03 a**	**0.43 ± 0.04 b**	**0.38 ± 0.02 b**
Cu (mg/Kg)	5	**0.42 ± 0.03b**	**0.65 ± 0.08ab**	**0.87 ± 0.07a**
	10	**0.42 ± 0.04c**	**0.66 ± 0.06b**	**0.93 ± 0.08a**
	25	**0.38 ± 0.03b**	**0.79 ± 0.15a**	**0.88 ± 0.06a**
	50	**0.30 ± 0.03c**	**0.51 ± 0.05b**	**0.72 ± 0.04a**
	Mean	**0.38 ± 0.03c**	**0.65 ± 0.06b**	**0.85 ± 0.06a**
Fe (mg/Kg)	5	88.08 ± 8.66	99.30 ± 9.02	78.82 ± 6.85
	10	85.81 ± 9.51	73.86 ± 4.51	64.89 ± 2.96
	25	58.52 ± 2.98	55.72 ± 1.90	48.86 ± 3.01
	50	23.95 ± 1.65	24.80±1.36	21.43 ±1.36
	Mean	64.09 ± 4.90	62.01 ± 2.60	53.50 ± 2.69
Zn (mg/Kg)	5	1.52 ± 0.17	1.41 ± 0.24	0.98 ± 0.09
	10	1.27 ± 0.18	1.09 ± 0.18	0.87 ± 0.14
	25	1.35 ± 0.23	1.25 ± 0.19	0.87 ± 0.23
	50	1.37± 0.33	1.26 ± 0.28	0.95 ± 0.34
	Mean	1.38 ± 0.20	1.24 ± 0.17	0.92 ± 0.18
pH	5	**4.67 ± 0.08b**	**4.89 ± 0.04ab**	**5.07 ± 0.04a**
	10	**4.71 ± 0.09b**	**5.07 ± 0.10a**	**5.16 ± 0.06a**
	25	**4.89 ± 0.07b**	**5.35 ± 0.07a**	**5.37 ± 0.07a**
	50	**5.16 ± 0.08b**	**5.50 ± 0.05a**	**5.48 ± 0.05a**
	Mean	**4.86 ± 0.08 b**	**5.20 ± 0.06 a**	**5.27 ± 0.04 a**

Table 4.2. Effects of vegetation type (V) and time on density (#seedlings/m^2) of initial seedlings, (N=1045), Cohort 1 recruits (N=840), Cohort 2 recruits (N=630), and Cohort 3 recruits (N=420). Effect size (Est.), standard errors (SE), and *P* values of a generalized linear mixed model with a Poisson error structure with vegetation type as a fixed effect, and transect and time as random effects. *P* values less than 0.05 are in boldface. Effect sizes are shown in the form of differences (contrasts) between the mean of cerrado denso and the mean of c.s.s and c.sujo, and between the mean of Time1 (first census) and the mean of the other times (census)

	Initial seedlings				**Cohort 1**				**Cohort 2**				**Cohort 3**			
Parameter	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*	Est.	SE	*z*	*p*
Intercept	1.52	0.09	16.73	**<0.001**	1.12	0.09	12.93	**<0.001**	0.21	0.17	1.25	0.21	0.08	0.11	0.74	0.45
V (c.s.s)	0.01	0.04	0.39	0.69	0.17	0.05	3.23	**0.001**	-0.45	0.11	-4.18	**<0.001**	-0.43	0.14	-3.96	**0.001**
V (c.sujo)	-0.05	0.04	-1.41	0.15	-0.42	0.06	-6.92	**<0.001**	-0.60	0.11	-5.48	**<0.001**	-0.56	0.14	-3.96	**<0.001**
Time2	-0.16	0.05	-3.48	**<0.001**	-0.31	0.06	-4.98	**<0.001**	-0.13	0.10	-1.20	0.23	-0.22	0.12	-1.89	0.059
Time3	-0.33	0.05	-3.48	**<0.001**	-0.41	0.06	-6.41	**<0.001**	-0.35	0.11	-3.17	**0.002**				
Time4	-0.42	0.05	-8.29	**<0.001**	-0.53	0.07	-8.02	**<0.001**								
Time5	-0.68	0.06	-12.19	**<0.001**												

Table 4.3. Effects of vegetation type (V), time and their interaction on richness (#species/m^2), density (#seedlings/m^2) and richness/density of recruited seedlings across time (N=630). Effect size (Est.), standard errors (SE), and *P* values of generalized linear mixed model with vegetation type as a fixed effect, and transect and time as random effects. *P* values less than 0.05 are in boldface. Effect sizes are shown in the form of differences (contrasts) between the mean of cerrado denso and the mean of c.s.s and c.sujo means, and between the mean of Time1 (first census) and the mean of the other times (census)

	Richness				**Richness/density**				**Density**			
Parameter	Est.	SE	*z*	*p*	Est.	SE	*t*	*p*	Est.	SE	*z*	*p*
Intercept	0.82	0.11	7.21	**<0.001**	1.40	0.20	6.88	**<0.001**	1.11	0.10	10.86	**<0.001**
V (c.s.s)	0.10	0.11	0.90	0.36	-0.72	0.21	-3.45	**<0.001**	0.12	0.09	1.29	0.19
V (c.sujo)	-0.21	0.11	-1.82	0.06	-0.64	0.21	-3.13	**0.002**	-0.30	0.10	-2.91	**0.003**
Time2	-0.65	0.13	-4.86	**<0.001**	-0.77	0.21	-3.71	**<0.001**	-0.89	0.12	-7.1	**<0.001**
Time3	-0.88	0.14	-6.12	**<0.001**	-0.99	0.21	-4.76	**<0.001**	-1.06	0.13	-7.90	**<0.001**
c.s.s*time2	-0.60	0.21	-2.84	**0.004**	-0.91	0.52	-1.73	0.08	-0.53	0.19	-2.73	**0.006**
c.sujo*time2	-0.39	0.21	-1.83	0.06	-0.70	0.52	-1.33	0.18	-0.27	0.20	-1.33	0.18
c.s.s*time3	-0.40	0.22	-1.84	0.06	-0.27	0.51	-0.52	0.59	-0.49	0.20	-2.40	**0.01**
c.sujo*time3	-0.31	0.23	-1.38	0.17	-0.37	0.51	-0.72	0.47	-0.20	0.21	-0.95	0.33

Table 4.4. Seedling limitation (proportion of subplots of $1m^2$ without seedlings of a particular species) for several woody species in cerrado denso, cerrado sensu stricto and campo sujo. A value of 1 indicates maximum limitation because seedlings of that particular species were not found in any of the subplots. Values are means ± SE (n=9) per species. Bold rows indicate species that differ significantly among vegetation types. For these species means with the same letter do not differ significantly according to Kruskal-Wallis pair-wise comparisons ($p<0.05$). Mean seedling limitations per site are also indicated.

COHORT 1	**cerrado denso**	**cerrado sensu stricto**	**campo sujo**
Aspidosperma macrocarpon	0.917± 0.036	1.000± 0.000	0.972± 0.018
Calliandra dysantha	0.861± 0.049	0.819± 0.056	0.847± 0.062
Campomanesia pubescens	**0.986± 0.014a**	**0.819± 0.056b**	**0.681± 0.075b**
Casearia altiplanensis	0.861± 0.053	0.792± 0.059	0.750± 0.059
Chamaecrista orbiculata	0.931± 0.022	0.944± 0.030	0.917± 0.021
Dalbergia miscolobium	0.875± 0.029	0.958± 0.021	0.931± 0.022
Erythroxylum campestre	0.625± 0.081	0.736± 0.053	0.708± 0.063
Eugenia bracteata	0.944± 0.022	0.819± 0.056	0.889± 0.033
Maprounea brasiliensis	0.986± 0.014	1.000± 0.000	0.958± 0.029
Miconia albicans	**0.681± 0.059b**	**0.806± 0.037ab**	**0.875± 0.055a**
Miconia fallax	**0.667± 0.047b**	**0.931± 0.037a**	**0.903± 0.035a**
Myrsine guianensis	**0.806 ± 0.052b**	**0.500± 0.083c**	**0.958± 0.021a**
Ouratea floribunda	0.861± 0.025	0.889± 0.044	0.847± 0.040
Roupala Montana	0.861± 0.025	0.889± 0.039	0.903± 0.046
Rourea induta	**0.903 ± 0.028b**	**0.986± 0.014a**	**0.958± 0.021ab**
Styrax ferrugineous	0.889 ± 0.033	0.958± 0.021	0.972± 0.018
Mean	0.85 ± 0.16	0.87 ± 0.18	0.88 ± 0.15

COHORT 2	**cerrado denso**	**cerrado sensu stricto**	**campo sujo**
Aspidosperma macrocarpon	0.972 ± 0.018	1.000±0.000	1.000± 0.000
Calliandra dysantha	0.792 ± 0.051	0.861±0.044	0.861± 0.033
Campomanesia pubescens	0.889± 0.039	0.792±0.086	0.861± 0.033
Casearia altiplanensis	0.819± 0.042	0.903±0.058	0.903± 0.058
Chamaecrista orbiculata	0.792± 0.047	0.903± 0.035	0.903± 0.078
Dalbergia miscolobium	**0.847± 0.046b**	**0.944± 0.030ab**	**1.000± 0.000a**
Erythroxylum campestre	0.806± 0.056	0.861± 0.044	0.903± 0.046
Eugenia bracteata	**0.958± 0.021a**	**0.764± 0.076b**	**0.958± 0.029a**
Maprounea brasiliensis	0.944± 0.022	0.986± 0.014	0.931± 0.042
Miconia albicans	0.889± 0.033	0.944± 0.030	0.903± 0.028
Miconia fallax	0.931± 0.030	0.958± 0.042	1.000± 0.000
Myrsine guianensis	**0.875± 0.050b**	**0.833± 0.055b**	**0.986± 0.014a**
Ouratea floribunda	**0.958± 0.029a**	**0.833± 0.036b**	**0.972± 0.018a**
Roupala Montana	**0.986± 0.014a**	**0.917± 0.029b**	**1.000± 0.000a**
Rourea induta	**0.917± 0.029b**	**1.000± 0.000a**	**0.972± 0.018a**
Styrax ferrugineous	0.958± 0.021	0.972± 0.018	0.986± 0.014
Mean	**0.90 ± 0.12b**	**0.90 ± 0.14b**	**0.94 ± 0.11a**

COHORT 3	**cerrado denso**	**cerrado sensu stricto**	**campo sujo**
Aspidosperma macrocarpon	1.000± 0.000	1.000± 0.000	1.000± 0.000
Calliandra dysantha	0.958± 0.021	0.931± 0.047	1.000± 0.000
Campomanesia pubescens	1.000± 0.000	0.986± 0.014	0.972± 0.018
Casearia altiplanensis	**0.792± 0.042b**	**0.986± 0.014a**	**0.972± 0.018a**
Chamaecrista orbiculata	0.986± 0.014	1.000± 0.000	0.986± 0.014
Dalbergia miscolobium	**0.917± 0.036b**	**0.986± 0.014ab**	**1.000± 0.000a**
Erythroxylum campestre	0.931± 0.030	0.861± 0.049	0.889± 0.039
Eugenia bracteata	1.000± 0.000	0.958± 0.021	0.972± 0.018
Maprounea brasiliensis	0.944± 0.030	0.986± 0.014	0.972± 0.018
Miconia albicans	**0.917± 0.029b**	**0.986± 0.014a**	**0.986± 0.014a**
Miconia fallax	0.903± 0.040	0.972± 0.018	0.972± 0.018
Myrsine guianensis	0.986± 0.014	0.944± 0.022	0.986± 0.014
Ouratea floribunda	**0.931± 0.022b**	**1.000± 0.000a**	**1.000± 0.000a**
Roupala montana	0.972± 0.018	0.917± 0.036	0.944± 0.022
Rourea induta	0.958± 0.029	1.000± 0.000	0.986± 0.014
Styrax ferrugineous	1.000± 0.000	0.986± 0.014	1.000± 0.000
Mean	**0.95 ± 0.09b**	**0.97 ± 0.07a**	**0.98 ± 0.05a**

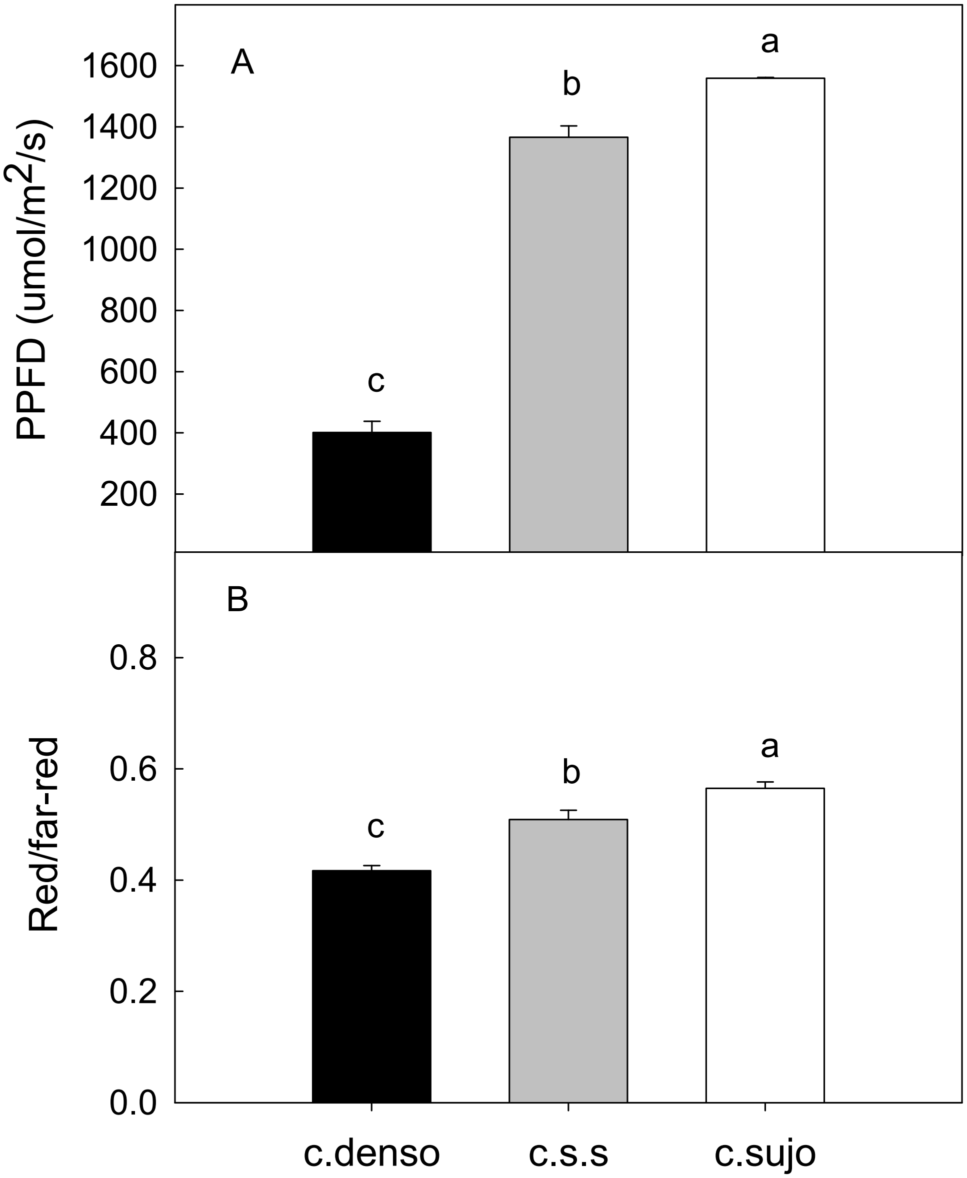


Figure 4.1. Photosynthetic Photon Flux Density (PPFD) (A), red/far-red wavelength ratios (B) in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo). Bars are means + SE. Bars with the same letter do not differ significantly ($p<$ 0.05) among vegetation types according to Tukey HSD post-hoc test. (n=90 for PPFD. n= 25 for red:far-red).

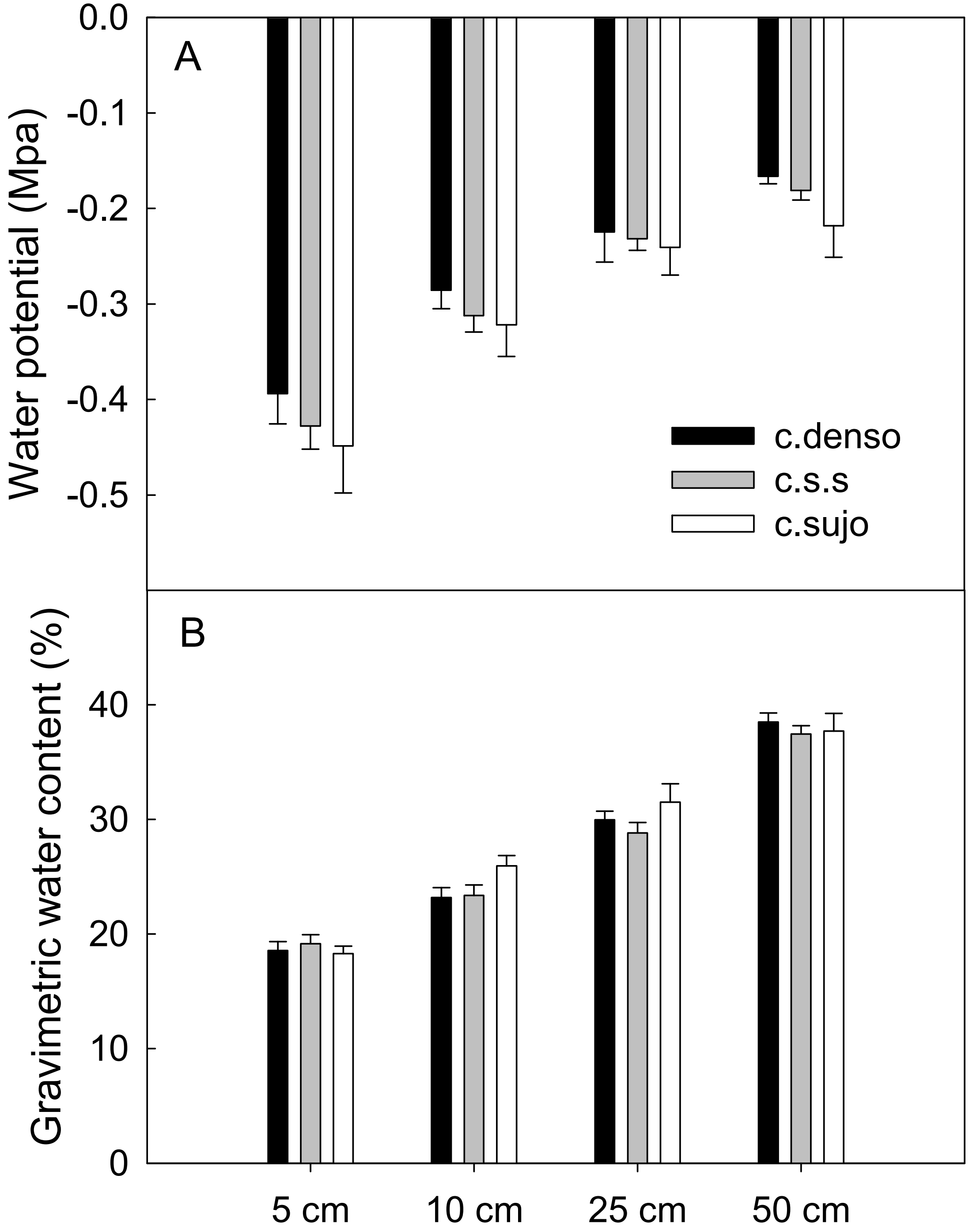


Figure 4.2. Soil water potential (A) and soil gravimetric water content (B) in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo) at 5, 10, 25 and 50 cm depth from the surface. Bars are means of the three transects in each vegetation type + SE (n=9); bars with the same letter do not differ significantly ($p < 0.05$) among vegetation types according to Tukey HSD post-hoc test.

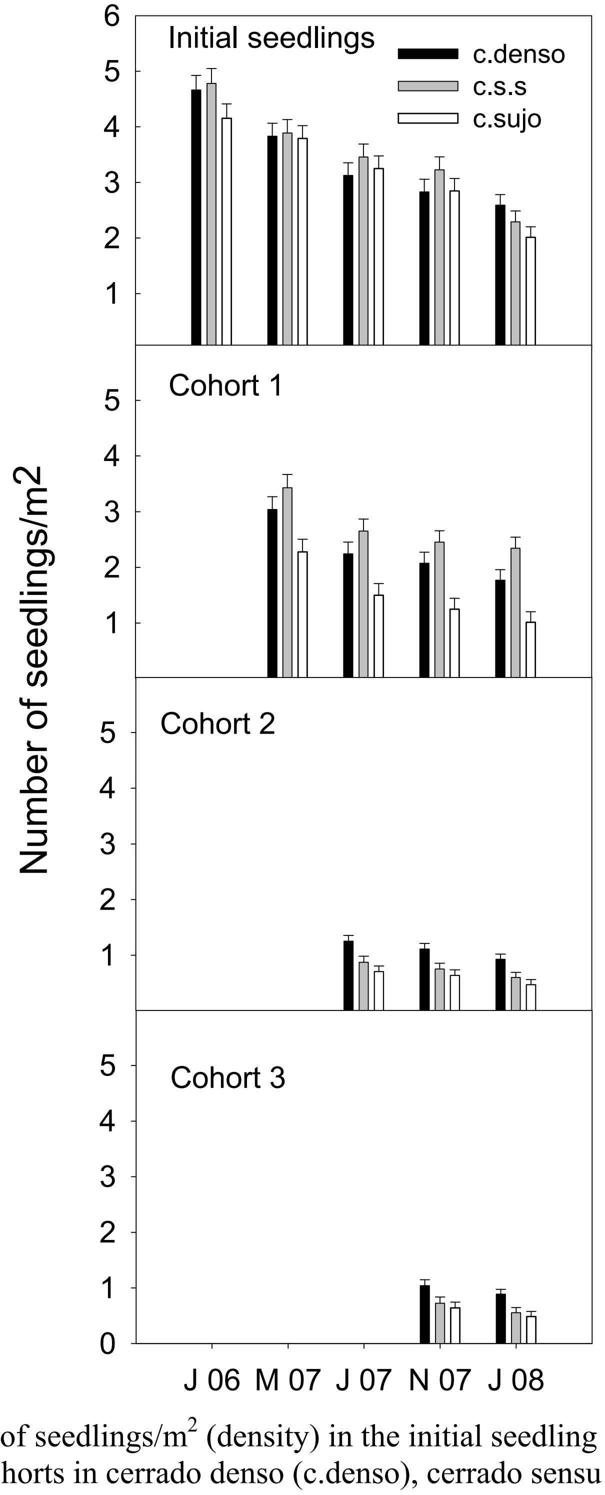


Figure 4.3. Number of seedlings/m^2 (density) in the initial seedling assemblage and the three consecutive cohorts in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo) during each census period: J 06 (July 2006), M 07 (March 2007), J 07 (July 2007), N 07 (November 2007), and J 08 (July 2008). Bars are means of 216 1m^2 subplots in the three transects per vegetation type + SE (n=72) during each census period.

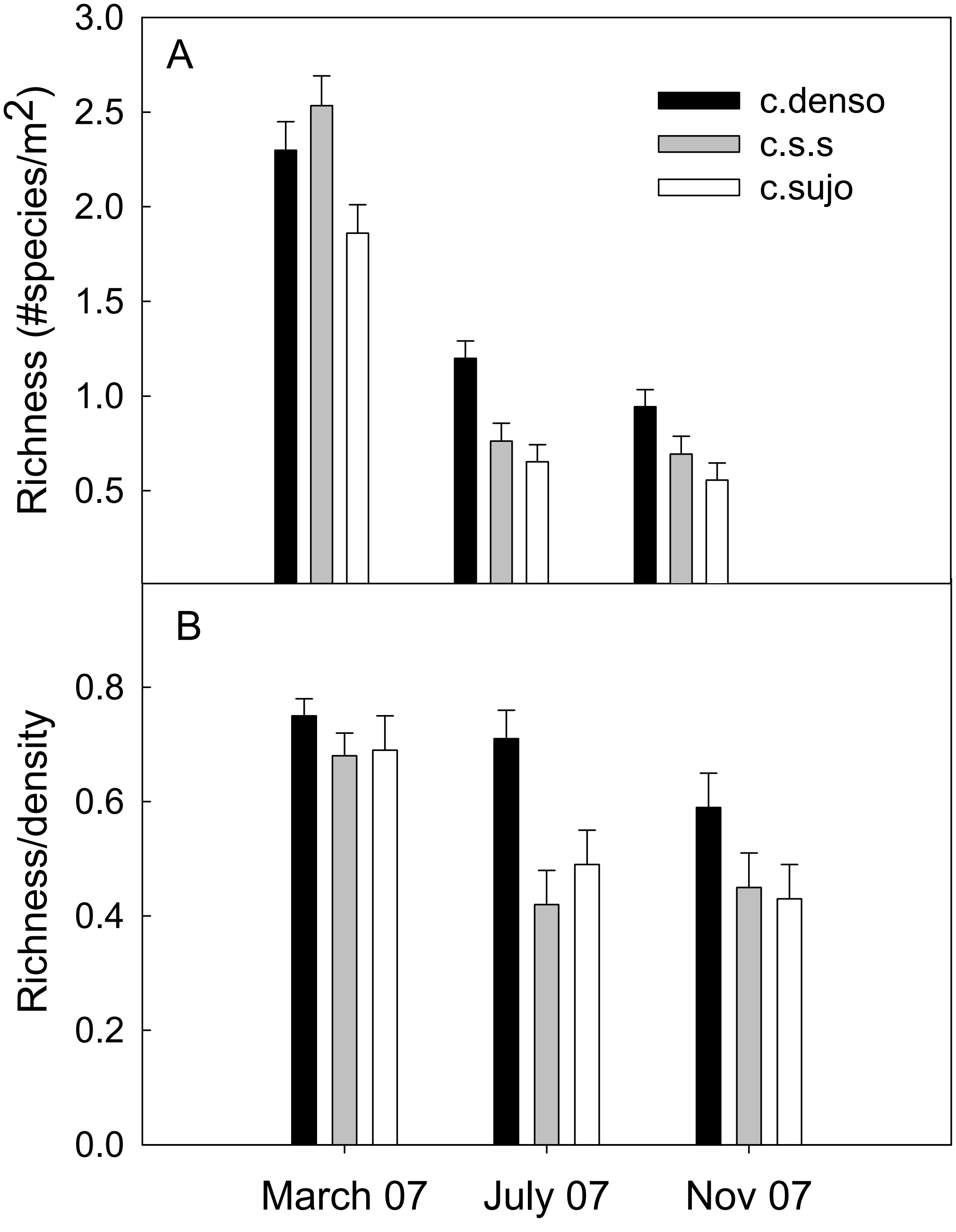


Figure 4.4. Richness (number of species/m^2) (A), and richness/density ratio of newly recruited seedlings (B) in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo) during each census period (March 2007, July 2007, and November 2007). Bars are means of 216 1m^2 subplots in the three transects per vegetation type + SE (n=72) during each census period.

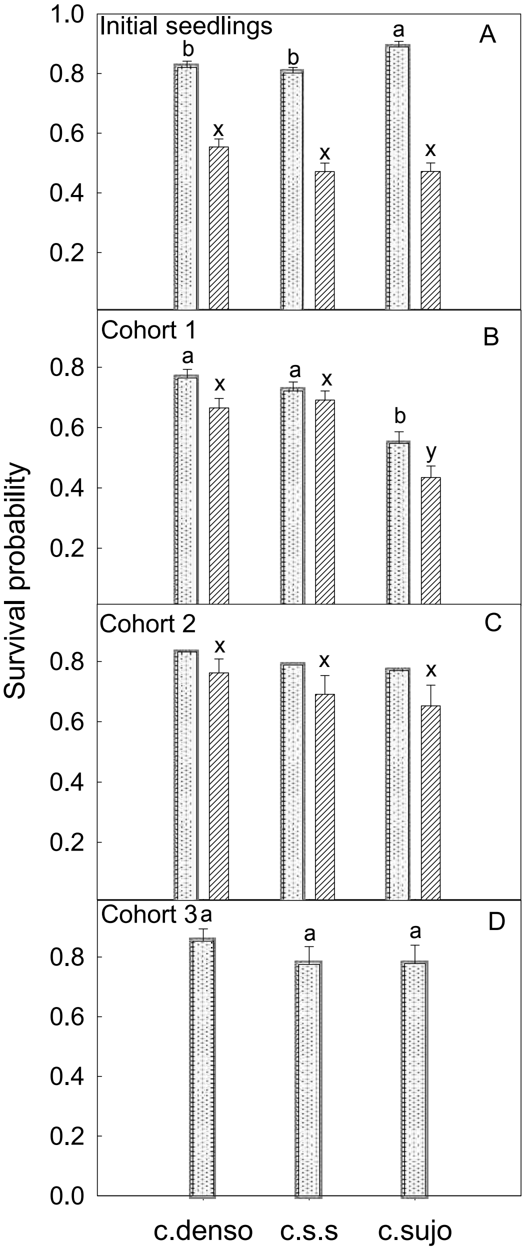


Figure 4.5. Mean (+ SE) survival probability (estimated by the Kaplan-Meier product-limit method) at 8 months (black bars) and at the end of the study period (white bars) for the initial seedling assemblage and for each cohort in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo). Study period for initial seedling assemblage was 24 months, for Cohort 1 was 16 months, for Cohort 2 was 12 months, and for Cohort 3 was 8 months. Bars of the same pattern topped with different letters differ significantly at $p< 0.05$ (pair-wise log-rank test comparisons). Survival probability at the end of 8 months study (black bars) of Cohort 2 were extrapolated from fitted linear equations (c.denso: $y = 1- 0.0007x$; c.s.s: $y = 0.98- 0.0008x$; c.sujo: $y = 1.02- 0.001x$) and therefore standard errors are not shown.

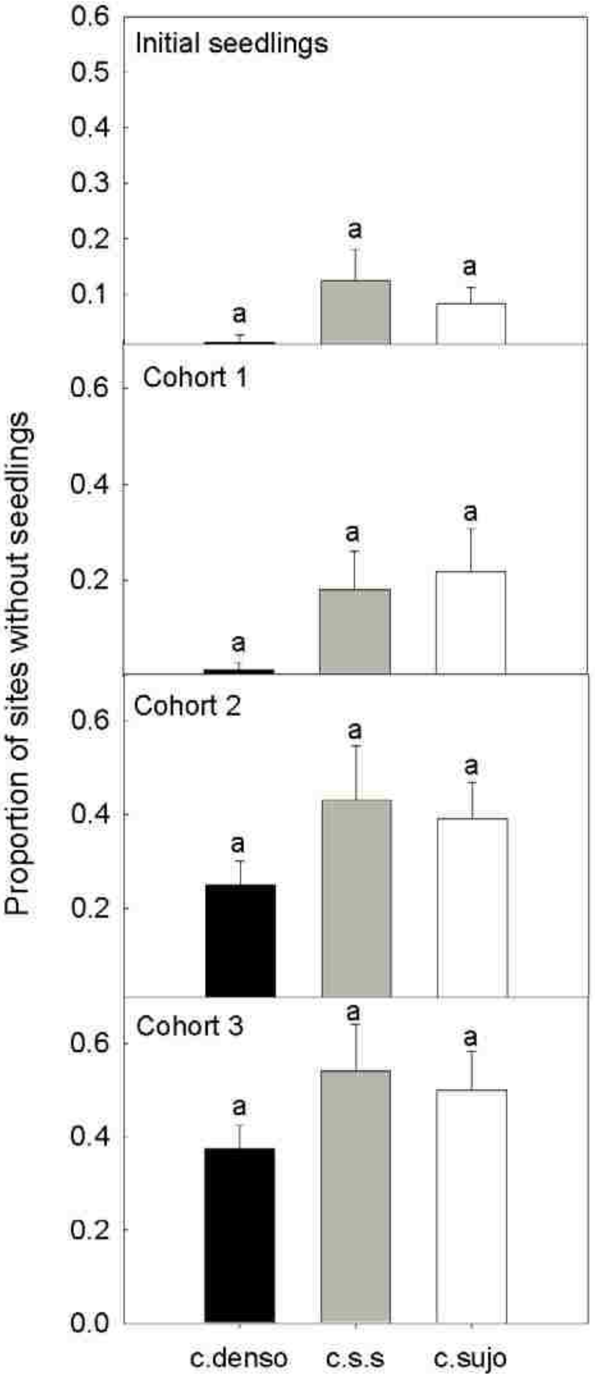


Figure 4.6. Proportion of sites (1m^{2}subplots) without seedlings in the initial seedling assemblage and the three consecutive cohorts in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo). The proportion is calculated as 1 minus the number of m^{2}subplots in each permanent 20 x 20 m plot where seedlings of woody species are found. Bars are means + SE (n=9). Means with the same letter do not differ significantly according to Kruskal-Wallis tests.

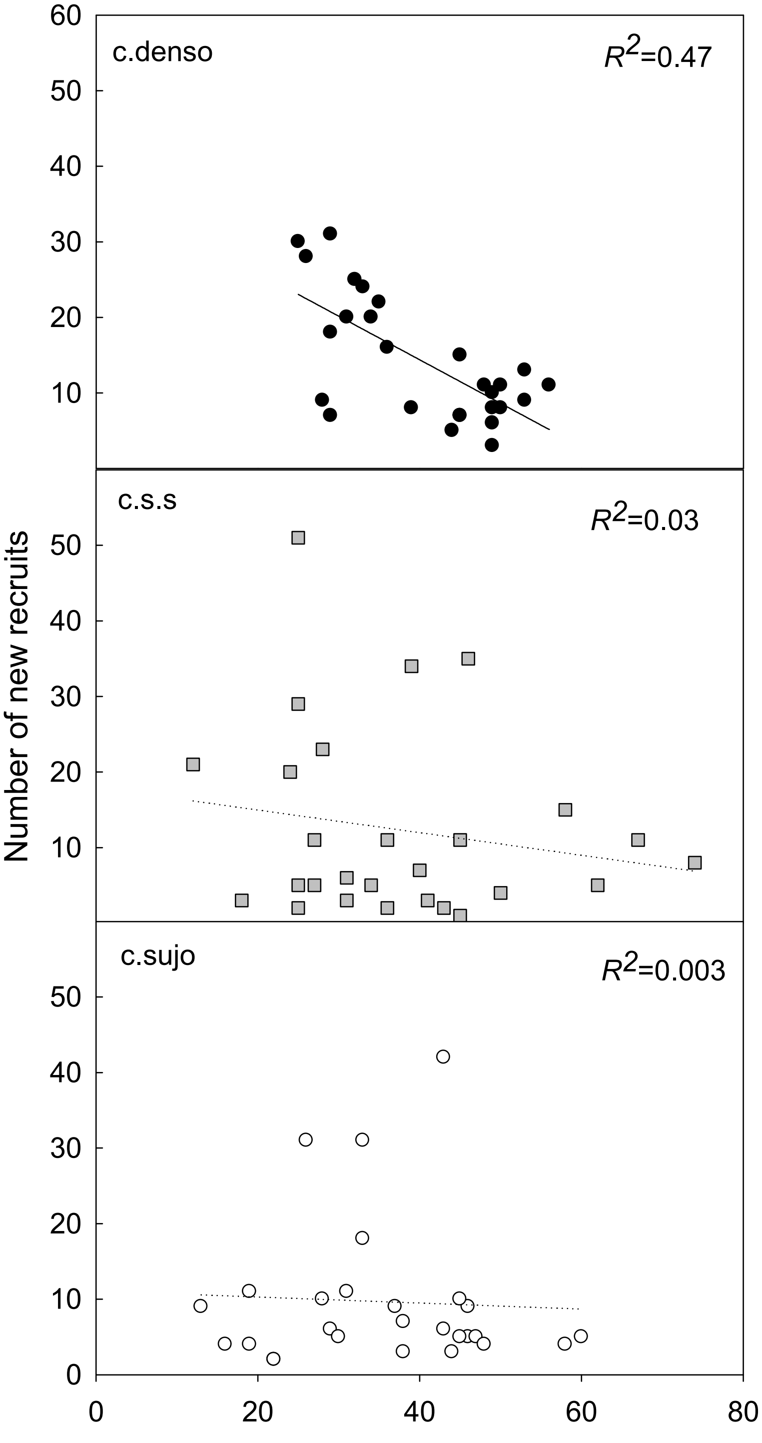


Figure 4.7. Number of new recruits as a function of number of pre-existing seedlings in each plot ($8m^2$) located in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo). Significant linear functions were found for c.denso (y = 37.45-0.58x, p<0.0001), but not for c.s.s (y = 17.96- 0.15x, p = 0.37) or for c.sujo (y = 11.08- 0.04x, p = 0.80).

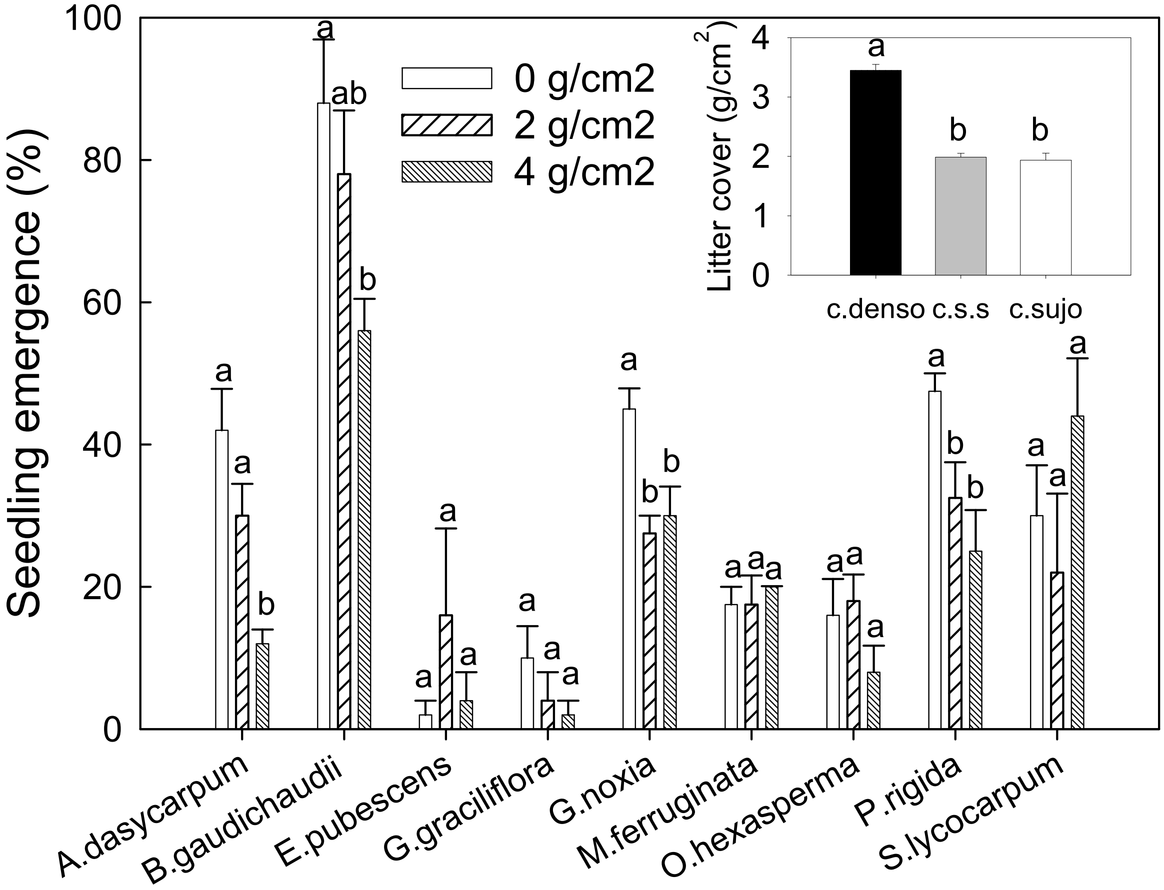


Figure 4.8. Seedling emergence (%) of nine cerrado woody species under different litter-cover (0g control, 2g/cm^{2,} 4g/cm^{2}) 4-5 weeks after sowing. Bars are means of five replicates of 20 seeds each + SE. Insert: surface litter cover collected in the field in cerrado denso (c.denso), cerrado sensu stricto (c.s.s) and campo sujo (c.sujo); bars are means of three transects in each vegetation type (n=27). Bars with the same letter do not differ significantly (p< 0.05) among vegetation types according to Tukey HSD post-hoc test.

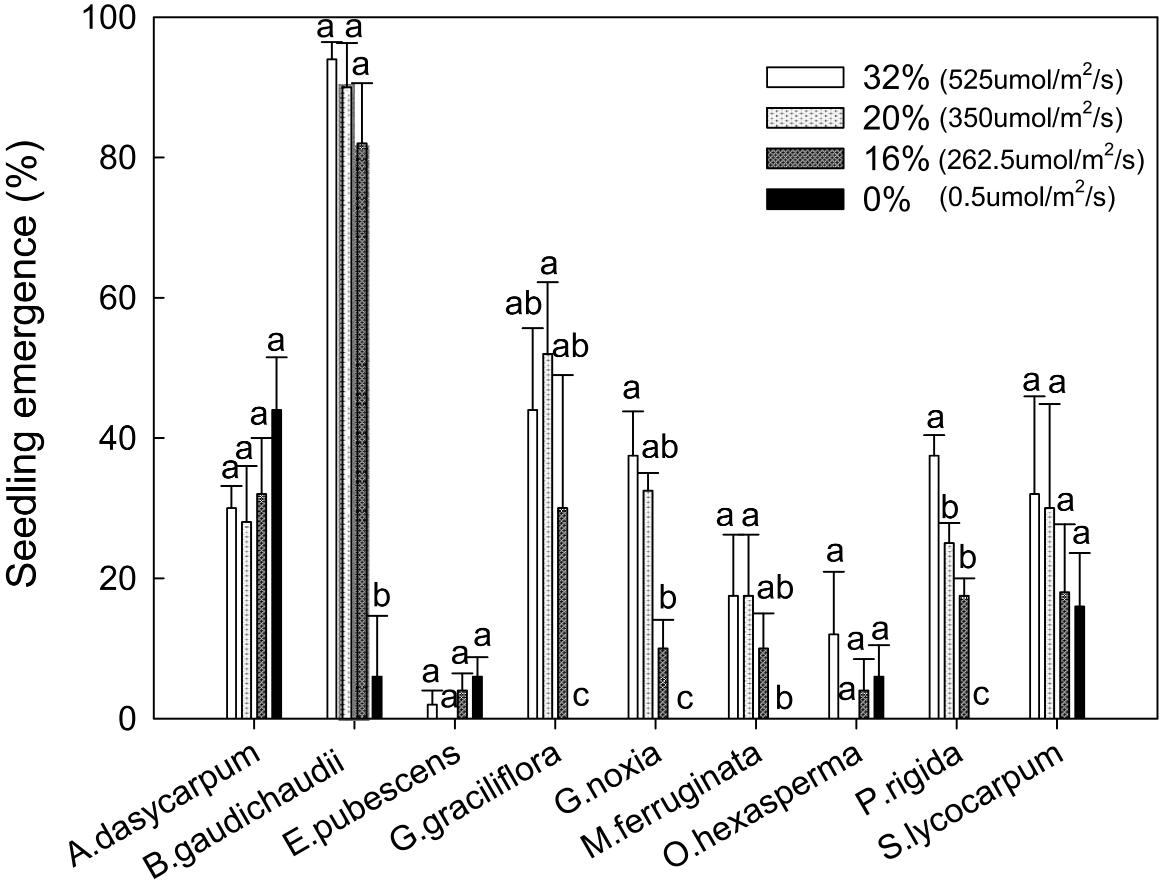


Figure 4.9. Seedling emergence (%) of nine woody species under four different light levels (30% control; 20% shade, 16% shade and 0% full sun light) 4-5 weeks after seed sowing. Bars are means of five replicates of 20 seeds each + SE; bars with the same letter do not differ significantly (*p*< 0.05) among vegetation types according to Tukey HSD post-hoc test.

Chapter 5

Fire reduces seedling establishment, increases vegetative reproduction and changes species composition of seedlings in the tropical savannas of central Brazil

Fire is a major disturbance factor in many ecosystems, particularly in tropical savannas around the world (Sarmiento 1984; Bond and van Wilgen 1996). The tropical savannas of central Brazil (cerrado) are currently burned every 1 to 3 years during the peak of the dry season for land clearing and managing natural or planted pastures for cattle (Coutinho 1990; Hoffman 2002). Other anthropogenic burns include accidental fires that burn large areas still covered with natural vegetation (Mistry and Berardi 2005). Evidence from old charcoal particles (ca. 32,000 years before present) indicates that prior to human-made fires, natural fires occurred in these savannas (Ledru 2002) and thus, most cerrado species likely have evolved with a long history of periodic burning. The rapid human occupation of the cerrado region during the last years, however, has changed the natural fire regime (season and frequency of burning) with potential consequences for the vegetation structure and composition (Miranda et al., 2002).

Savanna plant species differ greatly in their tolerance to fire and in their capacity to recover subsequently (Gignoux et al., 2009; Gignoux et al., 1997). Some may resist fire; others may be able to sprout from underground organs, while others may have to recover by seed germination (Bond and van Wilgen 1996). Most cerrado trees and large shrubs, for instance, resist fire because their highly suberized trunks and branches insulate living tissues from high fire temperatures (Miranda et al., 2002). After the passage of fires, many cerrado herbaceous and woody species are able to resprout from root buds and subterranean organs such as rhizomes, bulbs, and xylopodia (Hoffman and Solbrig 2003). While some species depend on fires to complete their life cycles and maintain or expand their population sizes (Hoffman 1998; Gottsberger and Silberbauer-Gottsberger 2006), others are negatively affected by fires. Sexual reproduction of many herbaceous species, for example, depends upon fire as it seems to stimulate intense flowering a few days or weeks after fires (Miranda et al., 2002). In contrast, sexual reproduction of many tree species decreases after fires because of reductions of plant size or size-specific seed production (Hoffmann and Moreira 2002). Frequent fires have been shown to decrease density and floristic diversity of fire-sensitive tree species (Durigan et al 1994; Moreira 2000, Nefabas and Gambiza 2007) while favoring species that are able to resprout from underground mersistems (Oliveira and Silva 1993; Hoffman 1998; Setterfield 2002). As a result of such species-specific differences in fire tolerance, frequent fires may potentially influence the structure and species composition of tropical savanna ecosystems (Moreira 2000).

In several fire-prone ecosystems, heat from fires has been shown to stimulate seed germination, particularly in species with impermeable seed coats (Baskin and Baskin 1998; Overbeck et al., 2006). Fire-triggered germination is the result of either heat shock or chemical products of combustion, and species appear to utilize one or another of these germination cues as an indication of relatively competition-free sites for seedling establishment (Keeley & Fotheringha 1998). Studies evaluating the effects of fire on seed germination of cerrado woody species are inconsistent; while some studies have shown positive effects of heat shocks of 70-100°C on seed germination of several woody species, in particular those with hard seed coats (Heringer 1971), other studies have shown negative or no significant effects of high temperatures on seed germination of some cerrado tree species (Rizzini 1976, Felippe and Silva 1984).

Predicting long-term effects of current fire regimes on cerrado woody plants requires studies on the effects of burning on seedling establishment in natural field conditions. Experiments testing the effect of heat on germination of several cerrado woody species are required to increase our understanding of vegetation-fire dynamics in tropical savannas as well as to develop new sustainable management tools.

The objective of this study is to evaluate the effect of fire on recruitment of seedlings and vegetative reproduction of cerrado species, and to evaluate the effects of heat-shocks on seed germination of 5 common cerrado tree species. I tested the following hypotheses: (1) Richness and density of seedlings is higher in fire-protected savannas than in accidentally burned savannas; (2) In accidentally and frequently burned savannas the number of seedlings is lower than the number of vegetative sprouts; (3) Seedling species composition differs among fire-protected, accidentally burned and frequently burned savannas; and (4) heat-shocks affect seed germination of cerrado woody species.

Methods

Study site

This study was conducted from July 2007 to July 2008 at the Instituto Brasileiro de Geografia e Estatística (IBGE) reserve, a field experimental station located 35 Km south of Brasilia, Brazil (15˚ 56' S, 47˚ 63' W, altitude 1100 m). Mean monthly temperatures range from 19˚C to 23˚C. Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August are often completely rainless. Average relative humidity during the rainy season is about 80% and drops to 55% during the dry season when daily minimum relative humidity may reach values as low as 10%. Fires are common during the dry season, particularly from August to September, when low relative humidity and dry grass-biomass increases the risk of fire propagation. The soils are very deep, well-drained, red or yellow clay-rich oxisols; structurally strong but nutrient poor. The IBGE reserve contains all major vegetation types of savannas from closed to very open savannas. Cerrado denso is a semi-closed woodland (40%-60% tree crown cover) with a moderately tall (usually 7 m) tree canopy. Because of the shade created by the canopy trees, the herbaceous and semi-woody ground vegetation has a lower cover than in relatively open cerrado vegetation. Cerrado sensu stricto is a savanna dominated by trees and shrubs (canopy generally less than 7 m tall) with 10 to less than 40% tree crown cover. Campo sujo has few short trees and scattered shrubs over a nearly continuous grass layer (the woody layer usually covers less than 10% of the ground).

Fire-treatments

In June 2005, I established one transect of approximately 1000 m in length in the reserve, spanning the three major vegetation types (cerrado denso, cerrado sensu stricto and campo sujo). This transect was placed along a continuous topographic gradient ranging from 1126 to 1143 m a.s.l., in vegetation types that have been protected from fire for at least 30 years and therefore comprise fire-protected savannas. I established 9 plots of 20 x 20 m along this transect (three per vegetation type). I determined preliminary species-area curves for cerrado denso and campo sujo areas, and the results indicated that about 85% of the maximum richness was attained at 1024 m^2.

In September 2005, a large accidental fire spreading from a nearby reserve burned approximately half the IBGE reserve including another transect I had established (of approximately 1000 m in length) spanning the three major vegetation types mentioned above. This transect also had been placed along a continuous topographic gradient ranging from 1117 to 1153 m a.s.l., in vegetation types that had been protected from fire for at least 30 years. Following the 2005 fire, the plots were easily identified because the locations of these plots had been mapped and marked. Our accidentally burned savannas comprise these plots.

A long term experimental fire project has been conducted at the IBGE reserve since 1992 (Sato 2003). This experimental fire project includes plots of 500 x 200m, burned every two years at the end of the dry season (end of September), and located in cerrado sensu stricto vegetation. Prior to these experimental burns, the plots had been protected from fire for 18 years (Cirne 2002). In this study, these plots are referred to as the frequently burned savanna.

Seedlings in fire-protected savannas

To quantify richness and density of seedlings of woody and herbaceous species in fire-protected savannas, in July 2007 I identified all seedlings up to 30 cm tall within 72 plots of 1m^2. The 1m^2 plots were randomly located within the 9 plots of 20 x 20 m (8 plots of 1m^2 per plot) previously established in the fire-protected savannas (cerrado denso, cerrado sensu stricto, and campo sujo). Twenty four plots of 1m^2 were established per savanna type. I performed preliminary species-plot curves in cerrado denso and campo sujo areas that indicated that about 90% of the maximum richness was attained using 8 plots of 1 x 1m.

Seedlings and sprouts in accidentally burned savannas and the frequently burned savanna

To quantify richness and density of seedlings and sprouts of woody and herbaceous species in accidentally burned savannas, I identified and measured (height) in July 2007 all seedlings and sprouts of woody and herbaceous species up to 30 cm tall within 72 plots of $1m^2$ (24 plots of $1m^2$ per savanna type). Eight plots of $1m^2$ plots were randomly established within each of the 9 plots of 20 x 20 m. The 20 x 20 m plots were temporary located in the savannas (cerrado denso, cerrado sensu stricto, and campo sujo) that were accidentally burned in September 2005.

To quantify richness and density of seedlings and sprouts of woody and herbaceous species in the frequently burned savanna, in July 2008 I identified all seedlings and sprouts of woody and herbaceous species up to 30 cm tall in 24 sub-plots of $1m^2$ randomly established within 3 plots of 20 x 20 m (8 subplots of $1m^2$ per plot). These 20 x 20 m plots were temporally located in the frequently burned savanna plot. The 500 x 200m plot had been burned for the last time in the end of September 2006.

Sprouts refer to new stems originating from buried root buds or rhizomes at some distance from the original parent stem. Sprouts originating from the base of roots or stems (root-crown and basal-stem sprouts, respectively) of adults were considered as a persistent mechanism rather than as a reproductive mechanism for extensive vegetative proliferation or clonal growth. Identification of seedlings and sprouts was made by comparison with herbarium specimens and with reference specimens collected from the field, and with help from local botanists. For each fire-treatment, within each vegetation type I calculated Shannon diversity index (H'): $H' = - \Sigma\, p_i\, lnp_i$; p_i is the proportion of species *i* relative to the total number of species.

Effects of heat-shocks on seed germination of woody species

To evaluate the effect of heat shocks on seed germination of cerrado woody species, I collected seeds from mature fruits of woody species that are widely distributed throughout much of the cerrado (Silva Júnior 2005). Seed viability percent was estimated by using 1% Tetrazolium red® solution (reference) and only species with an initial viability higher than 15% were included in the experiment. Seeds were cleaned by removing fruit tissues that normally are lost during dispersal. Prior to germination, seeds were placed in 90-mm diameter glass Petri dishes, then heated at 30, 60 and 90 °C in an oven for 5, 10 and 15 minutes, using a factorial experimental design for a total of nine heat-shock treatments. These treatments tried to simulate ranges of temperature-time periods that have been found at depths from 0 to 7 cm depth in cerrado soils during fires (Coutinho 1990, Miranda et al., 1993) or inside woody fruits of some species (Cirne 2002). Each treatment consisted of five replicates of 10 seeds, totaling 500 seeds per species. The treatments were compared with a control (seeds not exposed to heat). After being heat-shocked, seeds were sown in an artificial substrate (mix of 70% peat moss and 30% vermiculite) in germination trays. The trays were randomly distributed on greenhouse benches, under temperature conditions of 25-28ºC, 30% full sunlight, and watered daily with an automated sprinkler system to field capacity. Germination, defined as radicle emergence, was monitored every three days for 60 days. Germination percent was calculated from the number of germinated seeds at the end of the study period. The number of germinated seeds by unit time was estimated from a germination speed index (Maguire 1962) $GSI = G_1/N_1+G_2/N_2+\ldots G_n / N_n$, where G_1, G_2, G_n is the number of germinated seeds; N_1, N_2, N_n is the number of days after sowing. A high GSI value indicates a high speed of germination.

Data analyses

Differences in seedling richness and density of woody and herbaceous species in fire protected and accidentally burned savannas were examined with two-way ANOVAs with fire and savanna type as main factors. Data were previously transformed to fulfill the assumptions of normality by taking the square root and adding 0.5 to each original value. Tukey HSD post hoc tests were used for multiple comparisons of pairs of means. Differences in richness and density of seedlings and sprouts in accidentally burned savannas and in the frequently burned savanna also were examined with two-way ANOVAs with savanna type and reproductive strategy as main factors.

Non-metric multi-dimensional scaling (NMDS) ordinations were used to examine patterns of species composition in fire-protected, accidentally burned savannas and the frequently burned savanna. Ordinations were performed with PRIMER v5 (Clarke & Warmick 2001).The purpose of the ordinations is to represent the sites as points in a two dimensional space such that the relative distances among points reflect the relative dissimilarities of the sites. Similarities between every pair of the seven sites were calculated using Bray-Curtis similarity coefficients, after square-root transforming species abundances of every entry in the similarity matrix.

Analyses of similarities in species composition among fire-protected savannas, accidentally burned savannas and the frequently burned savanna were examined with an analogue of the 1 way-ANOVA (ANOSIM) under the null hypothesis that there are no species assemblage differences among fire-protected savannas, accidentally burned savannas and the frequently burned savanna. ANOSIM routines are permutation/randomization tests that assess differences between average ranks between and within groups. Values on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity between groups) reflect multiple pair-wise comparisons with groups well separated ($R > 0.75$), groups overlapping but differing ($R> 0.5$) or barely separated at all ($R <0.25$).

Differences in seed germination percent and speed of germination under different heat-shock treatments were also examined with two-way ANOVAs with heat-shock treatment and species as main factors. For each species, separate one-way ANOVA tests were performed to examine the effect of heat-shock treatments on seed germination. Tukey HSD post hoc tests were used for multiple comparisons of means. These analyses were performed with JMP 7 (SAS Cary, NC).

Results

The total number of species, abundance, and diversity of seedlings of woody species was higher than those of herbaceous species in all studied sites: fire-protected savannas, accidentally burned savannas, and the frequently burned savanna (Table 5.1). Total abundance of woody and herbaceous seedlings was higher in fire protected (363) than in accidentally burned savannas (198) and the frequently burned savanna (112) respectively. In accidentally burned savannas and the frequently burned savanna, total abundance of sprouts was higher (727) than total abundance of seedlings (310) for both woody and herbaceous species (Table 5.1). Sprouts were taller than seedlings in all accidentally burned savannas and in the frequently burned savanna (Table 5.2). However, significant differences in height between seedlings and sprouts were only found for woody species in accidentally burned cerrado denso, accidentally burned cerrado sensu stricto, and frequently burned cerrado sensu stricto (Table 5.2).

Richness and density of seedlings in fire-protected savannas and accidentally burned savannas

Fire, vegetation type, and their interaction had a significant effect on richness (2-way ANOVA, $F_{\text{fire (1, 138)}}= 7.43$, $p= 0.007$; $F_{\text{veg (2, 138)}}= 5.60$; $p=0.005$; $F_{\text{fire x veg (2, 138)}} = 3.12$, $p= 0.05$) and density (2- way ANOVA, $F_{\text{fire (1,138)}}= 7.33$, $p= 0.008$; $F_{\text{veg (2, 138)}}= 6.29$, $p= 0.002$; $F_{\text{fire x veg (2, 138)}}= 5.74$, $p = 0.004$) of woody seedlings in accidentally burned savannas (Figure 5.1AB). Richness and density of woody seedlings were significantly higher in fire-protected than in accidentally burned cerrado denso (Figure 5.1AB). However, richness and density of woody seedlings did not differ significantly between fire-protected and accidentally burned cerrado sensu stricto or between fire-protected and accidentally burned campo sujo (Figure 5.1AB). Richness and density of woody seedlings in fire-protected savannas was significantly lower in campo sujo than in cerrado denso and cerrado sensu stricto. Richness and density of woody seedlings, however, did not differ significantly among accidentally burned savannas (Figure 5.1AB).

Fire, vegetation type, and their interaction also had a significant effect on richness (2-way ANOVA: $F_{\text{fire (1, 138)}} = 54.95, p < 0.0001$; $F_{\text{veg (2, 138)}} = 4.74$; $p = 0.001$; $F_{\text{fire x veg (2, 138)}} = 8.55, p = 0.0003$) and density (2-way ANOVA, $F_{\text{fire (1,138)}} = 45.47, p < 0.0001$; $F_{\text{veg (2, 138)}} = 2.88, p = 0.05$; $F_{\text{fire x veg (2, 138)}} = 9.65, p = 0.0001$) of herbaceous seedlings in accidentally burned savannas (Figure 5.1CD). Richness and density of herbaceous seedlings were higher in fire-protected cerrado sensu stricto and fire-protected campo sujo than in accidentally burned cerrado sensu stricto, and accidentally burned campo sujo, respectively. However, richness and density of herbaceous seedlings did not differ significantly between fire-protected and accidentally burned cerrado denso (Figure 5. 1CD). Richness and density of herbaceous seedlings in fire-protected savannas was significantly lower in cerrado denso than in cerrado sensu stricto or campo sujo. Richness and density of herbaceous seedlings, however, did not differ significantly among accidentally burned savannas (Figure 5.1CD).

Seedlings and vegetative sprouts in accidentally burned savannas

In the accidentally burned savannas, reproductive strategy, but not vegetation type had a significant effect on richness (2-way ANOVA, $F_{\text{rep (1, 138)}} = 51.93, p < 0.0001$; $F_{\text{veg (2, 138)}} = 1.62$; $p = 0.20$; $F_{\text{rep x veg (2, 138)}} = 1.02, p = 0.36$) and density (2-way ANOVA, $F_{\text{rep (1,138)}} = 56.25, p < 0.0001$; $F_{\text{veg (2, 138)}} = 1.01, p = 0.37$; $F_{\text{rep x veg (2, 138)}} = 0.32, p = 0.73$) of woody recruits. In all three accidentally burned savannas, richness and density of woody sprouts were significantly higher than richness and density of woody seedlings (Figure 5.2AB). Neither richness nor density of woody seedlings and sprouts, however, differed among accidentally burned savannas (Figure 5.2AB).Reproductive strategy, but not vegetation type, also had a significant effect on richness (2-way ANOVA, $F_{\mathrm{rep}\ (1,\ 138)} = 32.03$, $p < 0.0001$; $F_{\mathrm{veg}\ (2,\ 138)} = 1.20$; $p=0.30$; $F_{\mathrm{rep\ x\ veg}\ (2,\ 138)} = 1.53$, $p= 0.22$) and density (2-way ANOVA, $F_{\mathrm{rep}\ (1,138)} = 25.78$, $p < 0.0001$; $\mathrm{F}_{\mathrm{veg}\ (2,\ 138)} = 2.66$, $p=0.07$; $F_{\mathrm{rep\ x\ veg}\ (2,\ 138)} = 2.48$, $p=0.09$) of herbaceous recruits in the accidentally burned savannas (Figure 5.2CD). In all three accidentally burned savannas, richness and density of herbaceous sprouts were significantly higher than richness and density of herbaceous seedlings (Figure 5.2CD). Richness of sprouts and seedlings of herbaceous species differed significantly in accidentally burned cerrado denso and accidentally burned campo sujo, but not in accidentally burned cerrado sensu stricto (Figure 5.2C) while density of sprouts and seedlings of herbaceous species differed significantly only in accidentally burned campo sujo (Figure 5.2D).

Seedlings and sprouts in accidentally and frequently burned cerrado sensu stricto savannas

When comparing accidentally and frequently burned cerrado sensu stricto savannas, it was clear that richness of woody recruits was significantly affected by reproductive strategy but not by fire type (Figure 5.3A; 2- way ANOVA, $F_{\mathrm{rep}\ (1,\ 92)} = 13.69$; $p=0.0004$; $F_{\mathrm{fire}\ (1,\ 92)} = 2.10$, $p=0.16$; $F_{\mathrm{fire\ x\ rep}\ (1,92)} = 0.002$, $p= 0.98$). Richness of woody sprouts was higher than richness of woody seedlings in accidentally burned cerrado sensu stricto but not in frequently burned cerrado sensu stricto savannas (Figure 5.3A). Density of woody recruits differed significantly between seedlings and sprouts and was significantly affected by fire type (Figure 5.3B; 2-way ANOVA, $F_{\mathrm{rep}\,(1,\,192)} = 30.60$; $p < 0.0001$; $F_{\mathrm{fire}\,(1,\,92)} = 7.30$, $p=0.008$; $F_{\mathrm{fire\ x\ rep}\ (1,92)} = 0.46$, $p = 0.50$). Density of woody sprouts was significantly higher than density of woody seedlings in both accidentally and frequently burned cerrado sensu stricto sites (Figure 5.3B).

Richness of herbaceous recruits also differed significantly between seedlings and sprouts and was significantly affected by fire type (Figure 5.3C; 2-way ANOVA, $F_{\mathrm{rep}\,(1,\,92)} = 13.04$; $p = 0.0005$; $F_{\mathrm{fire}\,(1,\,92)} = 24.12$, $p < 0.0001$; $F_{\mathrm{fire\ x\ rep}\ (1,\,92)} = 1.82$, $p = 0.19$). Richness of herbaceous sprouts was significantly higher than richness of herbaceous seedlings in frequently burned, but not in accidentally burned cerrado sensu stricto sites (Figure 5.3C). Although richness of herbaceous seedlings did not differ significantly between frequently burned and accidentally burned cerrado sensu stricto sites, richness of sprouts was significantly higher in frequently burned than in accidentally burned cerrado sensu stricto (Figure 5.3C).

The effect of fire type on density of herbaceous recruits was dependent on their reproductive strategy (Figure 5.3D; 2-way ANOVA, $F_{\mathrm{rep}\ 1,\,192} = 19.72$; $p < 0.0001$; $F_{\mathrm{fire}\ 1,\,192} = 30.38$, $p < 0.0001$; $F_{\mathrm{fire\ x\ rep}\ 1,192} = 7.49$, $p = 0.007$). Density of herbaceous seedlings did not differ significantly between frequently burned and accidentally burned cerrado sensu stricto sites. However, density of herbaceous sprouts was significantly higher in frequently burned than in accidentally burned cerrado sensu stricto savannas. Richness and density of herbaceous sprouts were significantly higher than richness and density of herbaceous seedlings in frequently burned, but not in accidentally burned cerrado sensu stricto (Figure 5.3CD).

Species composition of woody and herbaceous seedlings in fire-protected, accidentally burned savannas and the frequently burned savanna

Species composition of woody seedlings differed among fire protected, accidentally burned savannas and the frequently burned savanna (Figure 5.4A). The study sites partially overlapped in their woody seedling species composition but were statistically distinguishable in the 2-dimensional ordination space (ANOSIM; $R = 0.69$, $p = 0.03$). On average, the similarity in species composition between fire protected and accidentally burned savannas (average of the three pair wise comparisons i.e., c.denso protected vs. c.denso accidentally burned; c.s.s. protected vs .c.s.s accidentally burned, and c.sujo protected vs c.sujo accidentally burned) was 37.65%. The average similarity in seedling species composition of the three fire-protected savannas (average of the three pair wise comparisons) was 47.74%, while the average similarity in seedling species composition of the three accidentally burned savannas (average of the three pair wise comparisons) was 44.16%. The average similarity in seedling species composition of accidentally burned savannas and the frequently burned savanna (average of the six pair wise comparisons among the 4 burned savannas) was 46.77%.

Species composition of herbaceous seedlings also differed significantly among fire protected, accidentally burned savannas and the frequently burned savanna (Figure 5.4B). Vegetation types separated well in their species composition and were statistically distinguishable in the 2-dimensional ordination space (ANOSIM; *R* = 0.82, *p*= 0.01).The average similarity in species composition of herbaceous seedlings between fire protected and accidentally burned savannas (average of the three pair wise comparisons i.e., c.denso protected vs. c.denso accidentally burned, c.s.s. protected vs. c.s.s accidentally burned, and c.sujo protected vs. c.sujo accidentally burned) was 31.46%. The average similarity in species composition of herbaceous seedlings of the three fire-protected savannas (average of the three pair wise comparisons) 62.46% while the average similarity in seedling species composition of the three accidentally burned savannas (average of the three pair wise comparisons) was 37.84%. The average similarity in seedling species composition of the accidentally burned savannas and the frequently burned savanna (average of the six pair wise comparisons among the 4 burned vegetation types) was 22.44%.

Seedlings of *Campomanesia sp*., *Chamaecrista decresens* and *Miconia albicans* (woody species), and seedlings of *Croton sp* (herbaceous species), were present exclusively in all fire-protected savannas while seedlings of *Connarus fulvus* and *Cybianthus detergens* woody species were present exclusively in all burned (accidentally and frequently burned) savannas. Seedlings of *Erythroxylum campestre* and *Myrsine guianensis* woody species were present in all fire-protected and all burned (accidentally and frequently burned) savannas. Seedlings of woody species such as *Calliandra dysantha*, *Campomanesia pubescens*, *Roupala montana*, and *Rourea induta* were present in fire-protected, accidentally burned savannas, and the frequently burned savanna, but their abundance differed widely across them. Five herbaceous species (*Banisteropsis campestris*, *Irlbachia speciosa*, *Palicourea officinalis*, *Ruellia incompta* and *Vigna sp.*)

were only found in the frequently burned savanna. In contrast, only one herbaceous species (*Eriosema defoliatum*) was exclusively present in accidentally burned cerrado denso. *Licania dealbata* was only present in accidentally burned cerrado sensu stricto sites, while *Croton didrichsenii* and *Dalechampia caperonioides* were found only in accidentally burned campo sujo.

Effects of heat-shocks on seed germination of woody species

Seed germination across heat-shock treatments differed widely among species ranging from 24.60% in O*uratea hexasperma* to 71.00% in *Brosimum gaudichaudii* (Figure 5.5). Heat-shock treatment, species, and their interaction had a significant effect on seed germination percent (2 way-ANOVA, $F_{\text{treatment (9,190)}} = 19.33$, $p < 0.0001$; $F_{\text{species (4, 190)}} = 45.14$, $p < 0.0001$; $F_{\text{treatment x species (36,190)}} = 2.51$, $p < 0.0001$) indicating that the effect of heat-shocks on germination percent was species-specific. Mean germination percent of seeds with heat-shocks of 90°C for 5 minutes (38.75% ± 7.87), 10 minutes (17.08% ± 4.52) and 15 minutes (3.75%± 1.17) was significantly lower than the mean germination percent of seeds without heat-shocks (44.58% ± 6.01), and that of seeds with heat shocks of 30°C and 60°C (Figure 5.5). Compared to the control, heat-shocks of 30°C for 5 and 10 minutes increased germination percent of *Ouratea hexasperma* seeds. However, compared to the control, no heat-shock treatment significantly increased seed germination percent of the remaining 4 species (Figure 5.5).

Seed germination speed across heat-shock treatments also differed among species ranging from 0.38 in *Acosmium dasycarpum* to 3.02 in *Brosimum gaudichaudii* (Figure 5.6).

Heat-shock treatment, species, and their interaction had a significant effect on seed germination speed (2 way-ANOVA, $F_{\text{treatment } (9,190)} = 8.83$, $p < 0.0001$; $F_{\text{species } (4, 190)} = 86.10$, $p < 0.0001$; $F_{\text{treatment x species } (36,190)} = 2.24$, $p = 0.0003$) indicating that the effect of heat-shocks on germination speed was species-specific. Mean germination speed of seeds with heat-shocks of 90°C for 5 minutes (1.04 ± 0.26), 10 minutes (0.51 ±0.14) and 15 minutes (0.11± 0.04) was significantly lower than the mean germination speed of seeds without heat-shocks (1.43± 0.31) and that of seeds with heat shocks of 30° and 60°C (Figure 6). Compared to the control, heat-shocks of 30°C for 10 minutes enhanced germination speed of *Ouratea hexasperma* seeds. However, compared to the control, no heat-shock treatment significantly increased seed germination rate of the other species studied (Figure 5.6).

Discussion

Seedlings of woody and herbaceous species were more abundant and diverse in fire-protected savannas than in accidentally burned savannas and the frequently burned savanna, suggesting that fire highly constrains post-burn seedling recruitment of cerrado species. Some cerrado species exhibit mechanisms to protect seeds from burning (Cirne 2002) but many do not. Because most cerrado species fruit and disperse their seeds during the late dry season and early wet season (Oliveira and Silva 1993; Gottsberger and Silberbauer- Gottsberger 2006), fires that occur in late dry season are most likely to negatively affect seedling establishment of species that lack mechanisms to protect seeds. For example, 1 year after a prescribed fire, no seedlings of *Palicourea rigida* were found in cerrado sensu stricto areas that had been burned in the late dry season because fire consumed almost all of its fruits (Ramos 2004). Although there is a possibility that some seeds survive the passage of fire by forming temporary soil seed banks (Andrade 2002; Sato 2003), the poor seedling establishment following fire might explain why many cerrado species have not evolved protective fruits that ensure seed availability after fire (Hoffmann and Moreira 2002).

In fire-protected savannas, woody seedlings were more abundant and diverse in closed than in open savannas while herbaceous seedlings exhibited the opposite pattern (more abundant in open than in closed savannas). However, no such differences in density and richness were found among savanna types that had been burned accidentally two years before our measurements. Although fire reduced seedling richness and density in closed (cerrado denso), intermediate (cerrado sensu stricto) and open savannas (campo sujo), this reduction was statistically significant only in closed savannas. The strong effect of fire on closed savannas supports the view that fire is a major factor controlling tree density variations and tree-grass ratios in the cerrado. Gradual and progressive increase of woody vegetation after fire exclusion has been found in savannas of Africa (Swaine et al., 1992), Venezuela (Silva et al., 2001) and Brazil (Coutinho 1990; Moreira 2000). By allowing the establishment of fire-sensitive species, protection against fire for long periods of time could lead to an increase in diversity of cerrado woody species (Moreira 2000). In a separate study, I found that density and survival of woody seedlings was significantly higher in closed than in open savannas that have been protected from fire for at least 30 years. By providing protection against high irradiance, high temperature and water stress, canopy cover facilitates seedling establishment of woody species (Salazar et al, unpublished results). Therefore, the low seedling abundance in burned savannas could be the result of reductions in canopy cover. A reduced canopy cover could constrain seedlings establishment by exposing seeds and seedlings to higher temperatures and water stress due to increases in light and temperature caused by tree defoliation after the passage of fire (Bond and van Wilgen 1996; Hoffmann 1996). In addition to fire, other factors such as soil nutrient status (Furley 1999), soil moisture (Furley and Ratter 1988) and minimum distance of the water table from the soil surface (Villalobos-Vega unpublished results) determine patterns of species diversity and abundance of cerrado woody species, particularly in the low parts of the topographic gradients where the soil is inundated for part of the year but dries in the dry season.

In our study, richness and density of woody seedlings and sprouts were higher than richness and density of herbaceous seedlings and sprouts, particularly in intermediate and open burned savannas. Because most fires in the cerrado are surface fires that burn the grassy herbaceous layer of the vegetation (Moreira 2000), the high amount of accumulated grass biomass in intermediate and open savannas likely resulted in a differential effect of fire between plant functional groups. Castro and Kauffman 1998 found that fire consumed 84% of the total aboveground biomass in open savanna stands, and only 33 % in closed savanna stands near Brasilia. The great proportion of grass and herbaceous biomass that serve as fuel for fire propagation and expansion, particularly in open savannas, could explain the larger reduction of richness and density of herbaceous recruits in open than in closed burned savannas. Results of our study also indicate that accidental fires had stronger effects on recruitment of woody and herbaceous species than frequent fires that occur in the late dry season, at least in intermediate savannas (cerrado sensu stricto). Differences in fire behavior are probably because of differences in fuel structure, moisture content, and the total quantity of biomass consumed (Castro and Kauffman 1998). Under low moisture conditions and when the distribution of grasses is continuous, accidental fires potentially have the most rapid rate of spread and high flame height. Because accidental fires are usually difficult to control, propagate easily, and cause intense combustion of the vegetation (Coutinho 1990), seedling richness and density were probably lower in accidentally burned than in frequently burned cerrado sensu stricto. In addition, intense accidental fires can volatilize excessive amounts of nitrogen and other nutrients; destroy organic matter and change soil structure, thus increasing soil erosion (De ronde et al., 1990) and decreasing potential seedling establishment.

In accidentally burned savannas and in the frequently burned savanna, richness and density of vegetative sprouts were significantly higher than richness and density of seedlings suggesting that sprouting from roots and rhizomes is the major means of reproduction for both woody and herbaceous species after fires. Seedlings, which rely on few seed reserves and their ability to acquire new carbon from photosynthesis, may initially be at a disadvantage in resource poor environments compared to vegetative offspring that receive resources from their parent (Matlaga and Horvitz 2009). In addition, sprouts grow much faster than seedlings and can quickly reoccupy their own sites (Bond and Midgley 2001). In our study, I found that sprouts were taller than seedlings, particularly sprouts of woody species in closed and intermediate burned savannas. Hoffmann (1998) also found larger size and greater fire tolerance of root sprouts relative to seedlings in several cerrado woody species. The ability to initiate new shoots from protected buds has been viewed as an adaptation to select for regeneration after destruction of the shrub canopy by fire or other disturbances (Keeley 1992). The ability to resprout relies on stored reserves that are protected from disturbance effects (Bell et al., 1996). Because building up sufficient reserves takes time, newly germinated seedlings will be unable to resprout if they have not accumulated enough reserves (Gignoux et al., 2009) which would explain why in burned savannas sprouts were more abundant and taller than seedlings. Resources allocated to such reserves are diverted from other functions such as growth and leaf construction and thus without a prolonged fire-free interval seedlings of many tree species that reach sexual maturity at large size would not be able to reach a fire-tolerant size (Hoffmann and Moreira 2002). Therefore, in burned savannas it is expected that vegetative sprouts can contribute more to future population growth than seedlings because they are most likely to survive burning and reach sexual maturity (Hoffmann 1998) with consequences for the community structure and species composition (see below).

Seedling species composition

In our study, species composition of woody and herbaceous seedlings differed significantly among fire protected, accidentally burned savannas and the frequently burned savanna. The difference in floristic composition among sites suggests that fire can critically affect patterns of species composition of the savanna community by constraining seedling establishment of fire-sensitive species. Frequent fires can cause severe changes in the species composition of cerrado communities because only a few fire-tolerant species become dominant (Pivello and Coutinho 1996). Our results are consistent with this observation because seedlings of several woody species were restricted to fire-protected sites, likely because of their poor fire tolerance. Woody seedlings of *Campomanesia sp* (Myrtaceae), *Chamaecrista decresens* (Fabaceae) and *Miconia albicans* (Melastomataceae), and herbaceous seedlings of *Croton sp* (Euphorbiaceae) were present in all the three fire-protected savannas. However, seedlings of other species were restricted to a particular vegetation type. For example, seedlings of common cerrado tree species such as *Aspidosperma macrocarpon* (Apocynaceae), *Blepharocalyx salicifolius* (Myrtaceae), *Byrsonima cocolobifolia* (Malpighiaceae), *Dalbergia miscolobium* (Fabaceae), *Guapira cf. noxia* (Nyctaginaceae) and *Miconia fallax* (Melastomataceae) were only present in fire-protected cerrado denso. Four species of woody (trees and shrubs) seedlings were present only in fire-protected cerrado sensu stricto (*Byrsonima verbascifolia* (Malpighiaceae), *Esenbeckia pumila* (Rutaceae), *Eugenia bracteata* (Myrtaceae), *Neea theifera* (Nyctaginaceae) and *Ossaea congestiflora* (Melastomataceae)); and three species (Connaraceae sp., *Mimosa pseudoradula* (Fabaceae), and *Vochysia elliptica* (Vochysiaceae)) in fire-protected campo sujo. On the other hand, seedlings of *Connarus fulvus* (Connaraceae) and *Cybianthus detergens* (Myrsinaceae) woody species were present exclusively in all burned savannas (accidental and frequently burned) suggesting that fire might favor seedling establishment of these two species. Seedlings of *Erythroxylum campestre* and *Myrsine guianensis* woody species were present in both fire-protected, accidentally burned and frequently burned savannas which could suggests that these species may be highly fire-tolerant and can easily regenerate in post-burned environments. Morphological and anatomical studies of three *Erythroxylum* species showed that during the initial stages of seedling development, growth of the primary root is fast and extensive while growth of the aerial shoot system is slow (Alonso and Machado 2007). The fact that these species have below-ground buds that enable them to re-sprout and thus re-establish the aerial shoots following clipping or burning would explain why they are found in fire-protected and burned savannas. Differences in species composition between fire-protected and burned sites also could be the result of changes in soil properties after the passage of fire. Floristic composition between fire-protected sites and annually burned sites in central Brazil was highly correlated with changes in soil texture (the proportion of sand/clay) and the amount of organic matter (which decreased in burned sites; Silva and Batalha 2008). Our study confirms that fire favors clonal root-sprouting in neo-tropical savannas and also is consistent with other studies that show fire-induced shifts to dominance by clonal species in tropical deciduous forests of India (Saha and Howe 2003), temperate forests that are dominated by *Populus* (Gom and Rood 1999), and forest understories that experience regular freezes (Olmstead et al 1993).

Heat-shock treatments

Many plant species found in fire adapted communities have seeds which require heating or other means of seed coat scarification for germination (Tyler 1995). In our study, heat-shock treatments affected seed germination of woody species, but their effects were species-specific. Heat-exposure of 90°C for longer than 10 minutes significantly reduced seed germination in all studied species suggesting a negative effect of high temperatures

(>90°C) during relatively long periods of time on seed germination. It is possible that extremely high temperatures may have caused seed mortality or may have induced dormancy, which resulted in low germination in the studied species. Our results agree with those reported by Rizzini (1976) and by Felippe and Silva (1984) who found out that heat shocks of 100 °C for 10 minutes inhibited or reduced germination of twenty cerrado woody species. Considerable variation in temperature has been recorded in cerrado fires, ranging from 85°C above soil surface in a cerrado sensu stricto, to 840°C at 60 cm height in a campo sujo (Miranda et al 1993). Therefore, low germination of seeds located at ground level or above is expected in many woody species after the passage of fire, once they have experienced temperatures higher than 90°C. In our study, heat-shocks of 30°C for 5 and 10 minutes enhanced germination of *Ouratea hexasperma* seeds (compared to the control) while heat-shocks of 30 and 60°C for 5, 10 and 15 minutes did not significantly affect seed germination in the other four studied species. These results indicate that seeds of these species might tolerate temperatures of up to 60°C for 15 minutes but do not depend on heat for germination. Coutinho (1990) reported temperatures of 74, 47, 33 and 25°C in the soil at depths of 0, 1, 2, and 5 cm, respectively during cerrado fires. During a fire in the IBGE reserve, pulses of heat generated by the flames penetrated slowly into the soil with maximum temperatures (29-38°C) at 2 cm occurring 5-15 minutes after the passage of fire (Miranda et al., 1993). Our results indicate that seeds buried in the soil or near the soil surface are more likely to tolerate short-term soil heating than seeds above the soil surface. Germination of some species such as *O. hexasperma*, however, could be stimulated during soil heating. Although I did not find a stimulatory effect of heat on seed germination of most of the woody species studied, other fire-related cues such as smoke and other products of combustion (i.e., ash, volatile chemicals) could affect germination of cerrado woody species. Positive effects of smoke and charred wood on seed germination of several species have been found in fire-prone plant communities of the Mediterranean basin (Crosti et al., 2006), Australia (Dixon et al.,1995; Read et al., 2000; Thomas et al., 2007) and California chaparral (Keeley and Fotheringham 1998). The role of smoke in germination of cerrado woody species is unknown; studies testing the effect of smoke on seed germination of cerrado species are needed.

Overall, our results indicate that 1) seedling establishment of woody and herbaceous cerrado species is lower in accidentally burned savannas and in the frequently burned savanna than in fire-protected savannas suggesting that fire highly constrains seed germination of fire-sensitive species; 2) sprouting was the major means of regeneration for both woody and herbaceous species in accidentally burned savannas and the frequently burned savanna; 3) species composition of woody and herbaceous seedlings differed significantly in fire-protected savannas, accidentally burned savannas and the frequently burned savanna, with some species restricted to either fire-protected or burned savannas; 4) seeds of five common cerrado woody species tolerated temperatures of up to 60°C for 15 minutes but did not depend on heat pulses for their germination. Heat-exposure of 90°C for longer than 10 minutes significantly reduced seed germination in all the studied species. This study shows that because fire reduces post-burn seedling establishment of woody and herbaceous species but increases vegetative reproduction of some species, permanent or long-term shifts in species composition are expected under the present fire regimes in the cerrado.

Table 5.1. Number of species, abundance, and Shannon diversity index of seedlings and sprouts of woody and herbaceous species in fire protected, accidentally burned savannas: cerrado denso (c.denso), cerrado sensu stricto (c.s.s), campo sujo (c.sujo), and frequently burned cerrado sensu stricto. Frequently burned cerrado sensu stricto is burned every two years in the late season (September). Surveys were conducted two years after accidental and frequent burns. Values are totals of 24 plots of $1m^2$ in each vegetation type and fire-treatment.

Reproductive mode	**Growth Habit**	**Fire status**	**Vegetation**	**Number of species**	**Abundance**	**Shannon Diversity index**
Seedlings	*Woody*	Fire protected	c.denso	32	90	3.15
			c.s.s	23	101	2.64
			c.sujo	18	39	2.69
		Accidental burn	c.denso	19	44	2.62
			c.s.s	23	67	2.71
			c.sujo	16	55	1.99
		Frequent burn	c.s.s	27	88	2.83
	Herbaceous	Fire protected	c.denso	9	21	2.02
			c.s.s	6	52	1.56
			c.sujo	8	60	1.84
		Accidental burn	c.denso	4	17	1.12
			c.s.s	5	10	1.51
			c.sujo	4	5	1.33
		Frequent burn	c.s.s	11	24	2.21
Sprouts	*Woody*	Accidental burn	c.denso	27	117	2.84
			c.s.s	28	129	2.92
			c.sujo	29	118	2.69
		Frequent burn	c.s.s	43	187	3.22
	Herbaceous	Accidental burn	c.denso	8	36	1.56
			c.s.s	5	19	1.27
			c.sujo	7	39	1.35
		Frequent burn	c.s.s	18	82	2.30

Table 5.2. Comparison of (woody and herbaceous species) seedling and sprout height above the soil surface in accidentally burned vegetation types: cerrado denso (c.denso), cerrado sensu stricto (c.s.s), campo sujo (c.sujo), and frequently burned cerrado sensu stricto. Measurements were taken two years after accidental and frequent burns. Bold rows indicate significant differences in height (p<0.05) between seedlings and sprouts according to t tests.

Habit	**Vegetation type**	**Fire regimen**	**Seedling height (cm) ± SE**	**Sprout height (cm) ± SE**	***t* value**	**df**	***P* value**
Woody	**c.denso**	**accidental**	**12.08 ± 0.62**	**16.58 ± 0.94**	**3.97**	**158**	**<0.0001**
	c.s.s	**accidental**	**12.78 ± 0.75**	**15.95 ± 0.58**	**3.37**	**194**	**0.0009**
	c.s.s	**Frequent**	**12.04 ± 0.69**	**16.55 ± 0.53**	**5.16**	**271**	**<0.0001**
	c.sujo	accidental	14.75 ± 1.05	15.86 ± 0.58	1.32	164	0.19
Herbs	c.denso	accidental	19.25 ± 1.81	16.06 ± 1.20	1.49	51	0.14
	c.s.s	accidental	15.85 ± 2.34	16.58 ± 1.61	0.30	27	0.76
	c.s.s	Frequent	18.53 ± 1.53	19.38 ± 0.84	0.49	104	0.62
	c.sujo	accidental	13.66 ± 3.94	16.84 ±1.20	0.96	41	0.34

Figure 5.1. Number of species/m^2 of woody (A) and herbaceous(C) seedlings, and number of seedlings/m^2 of woody (B) and herbaceous (D) species in fire-protected (white bars) and accidentally burned (black bars) cerrado denso (c.denso), cerrado sensu stricto (c.s.s), and campo sujo (c.sujo).Bars are means + SE (n=24); bars topped with the same letter do not differ significantly (p< 0.05) among vegetation types according to Tukey HSD post-hoc test.

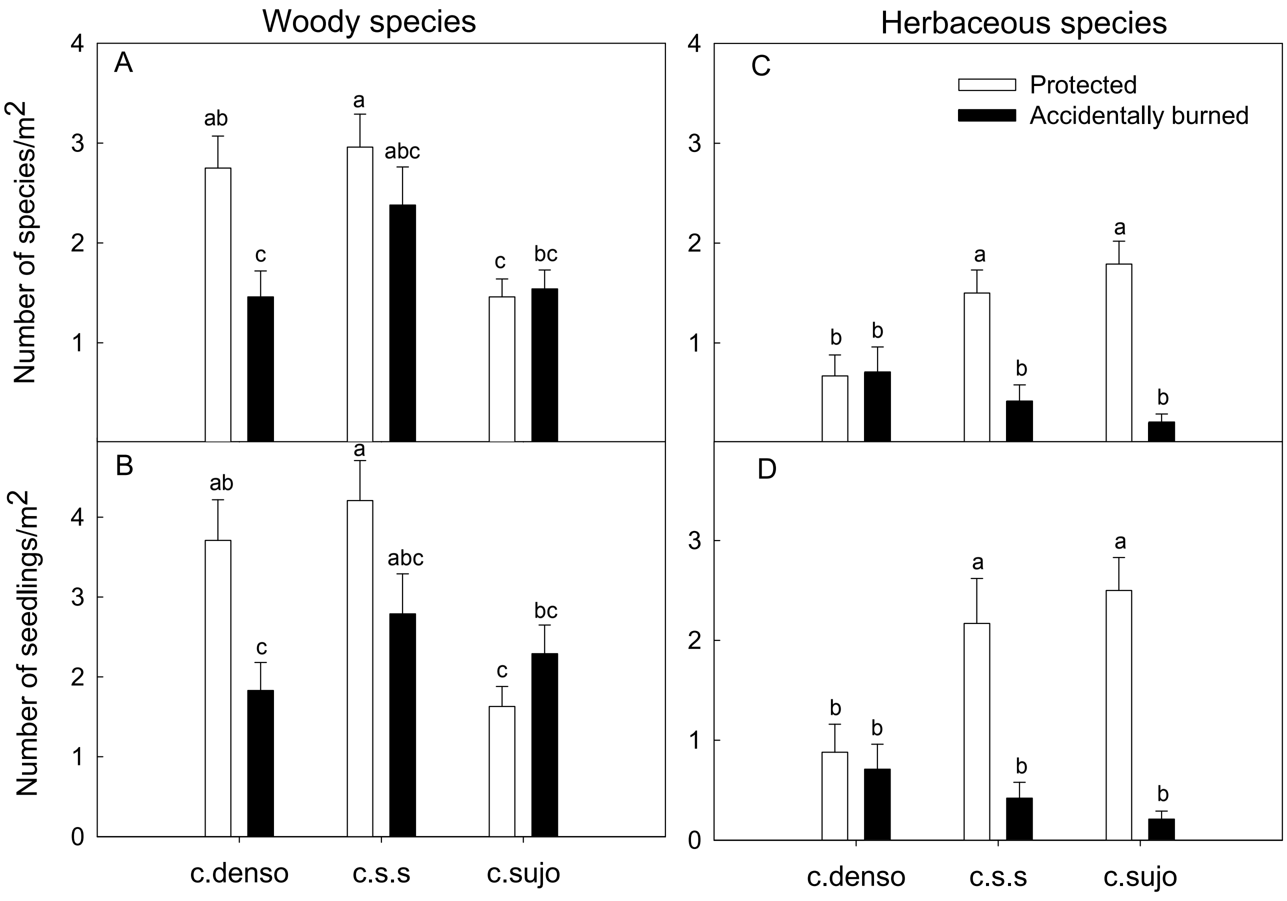


Figure 5.2. Number of species/m^2 of woody (A) and herbaceous(C) seedlings (dark gray bars) and sprouts (light gray bars), and number of individulas/m^2 of woody (B) and herbaceous (D) seedlings and sprouts in accidentally burned cerrado denso (c.denso), cerrado sensu stricto (c.s.s), and campo sujo (c.sujo). Bars are means + SE (n=24); bars with the same letter do not differ significantly (*p*< 0.05) among vegetation types according to Tukey HSD post-hoc test.

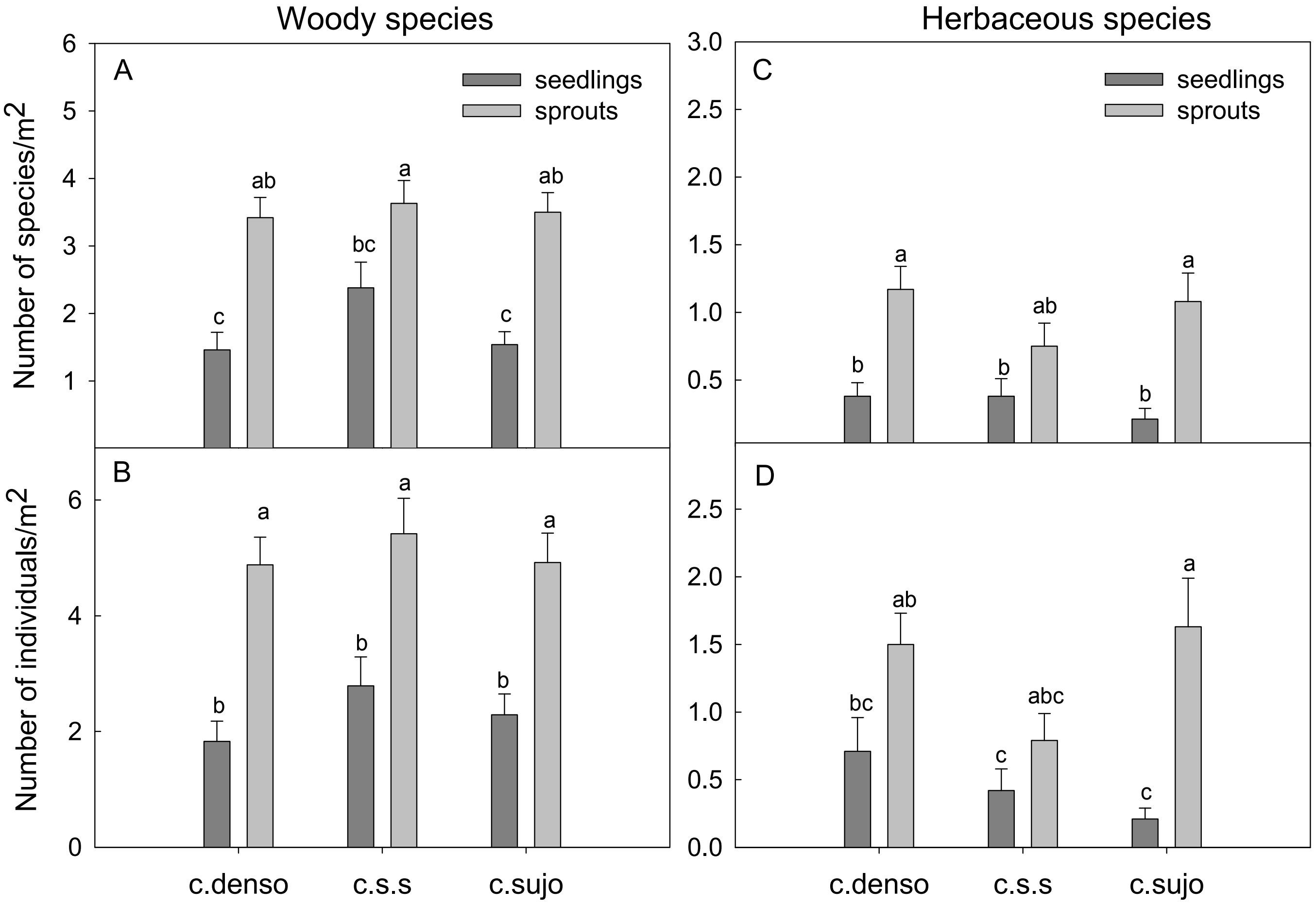
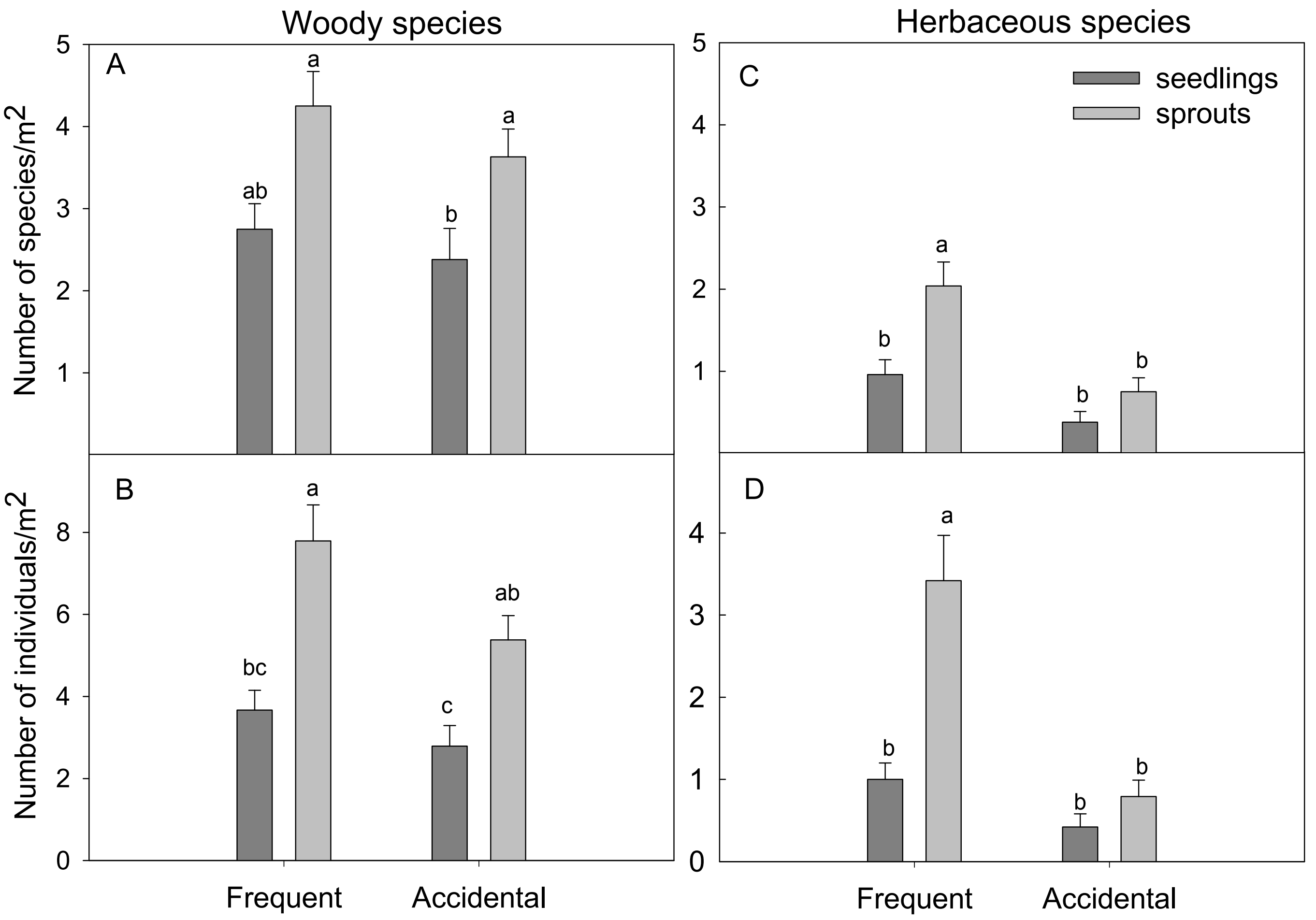


Figure 5.3. Number of species/m^2 of woody (A) and herbaceous(C) seedlings (dark gray bars) and sprouts (light gray bars), and number of individulas/m^2 of woody (B) and herbaceous (D) seedlings and sprouts in frequently burned and accidentally burned cerrado sensu stricto. Bars are means + SE (n=24); bars topped with the same letter do not differ significantly (p< 0.05) among accidentally and frequently burned cerrado sensu stricto according to Tukey HSD post-hoc test.



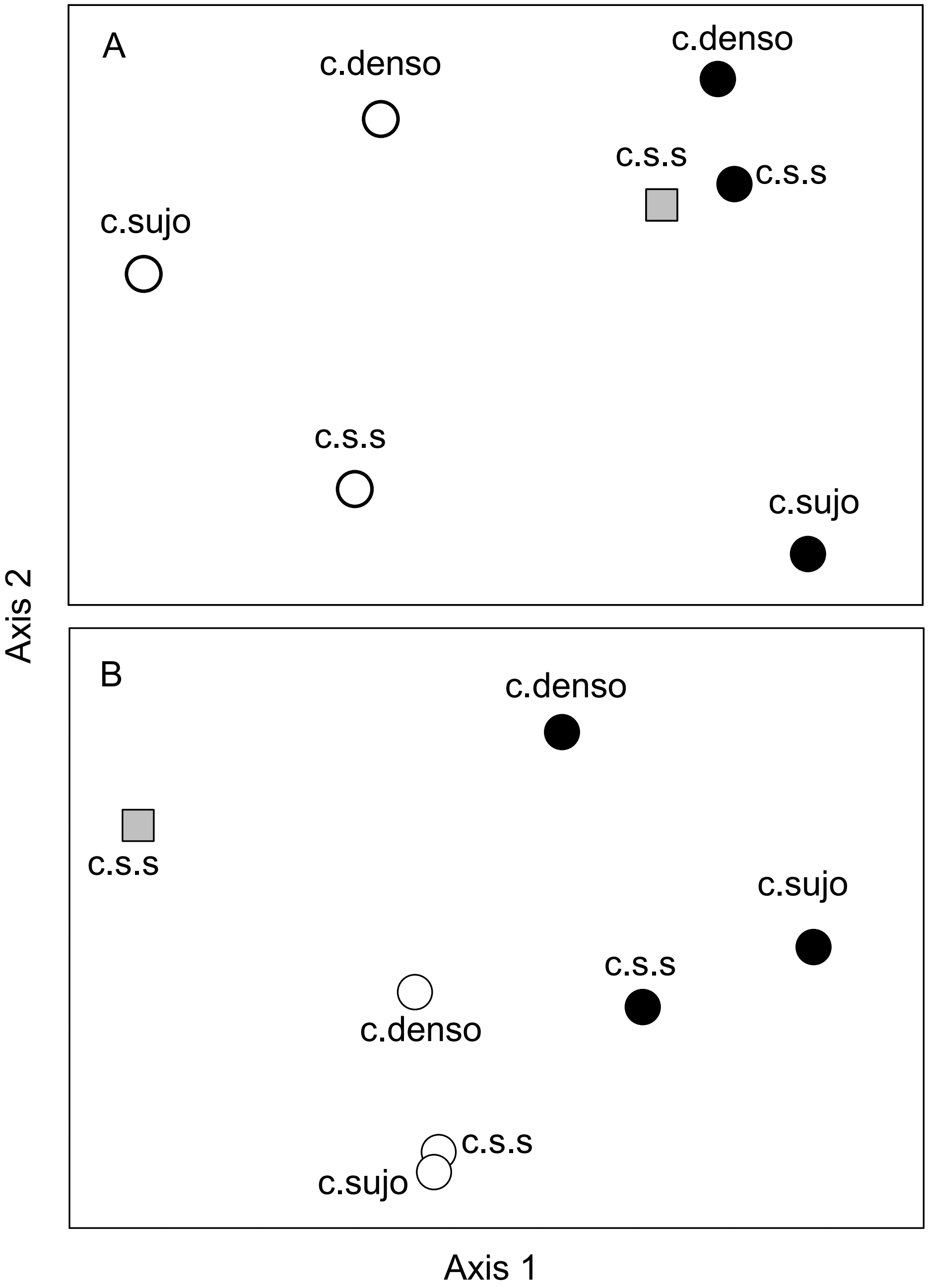


Figure 5.4. Non-metric multidimensional scaling (NMDS) ordination for the composition of seedlings of woody species (A) and for the composition of seedlings of herbaceous species (B) in fire-protected vegetation types (white circles), accidentally burned vegetation types (black circles), and frequently burned cerrado sensu stricto (gray square). c.denso, c.s.s and c.sujo correspond to cerrado denso, cerrado sensu stricto and campo sujo, respectively. Points that are close together represent sites with similar species composition; points that are far apart correspond to sites with very different species composition.

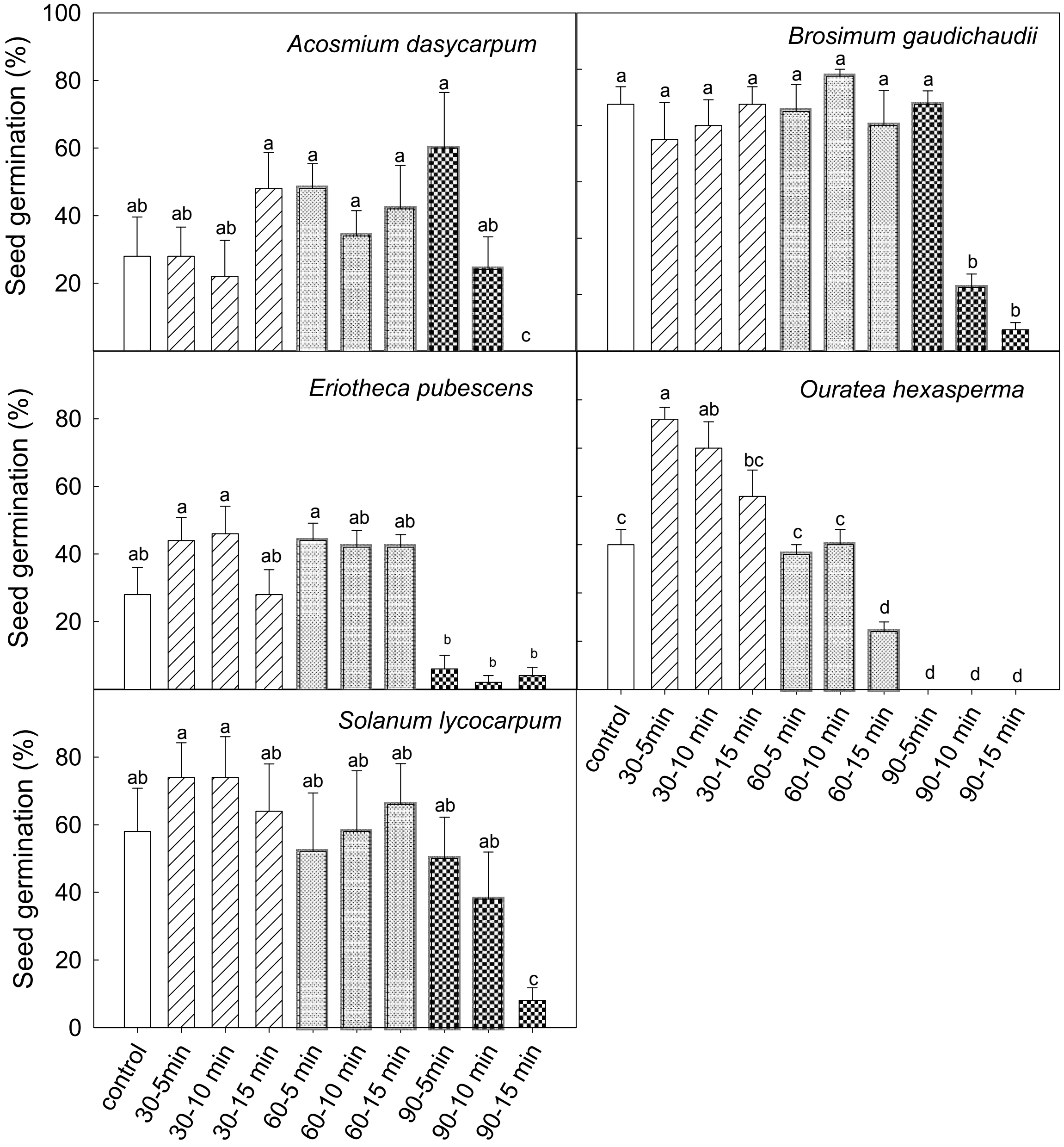


Figure 5.5. Seed germination (%) of five cerrado woody species under nine heat-shock treatments (30, 60 and 90°C for 5, 10, and 15 minutes each) plus control (seeds not exposed to heat-shocks) 60 days after seed sowing. Bars are means of five replicates of 10 seeds each + SE; bars topped with the same letter do not differ significantly ($p < 0.05$) among treatments according to Tukey HSD post-hoc test.

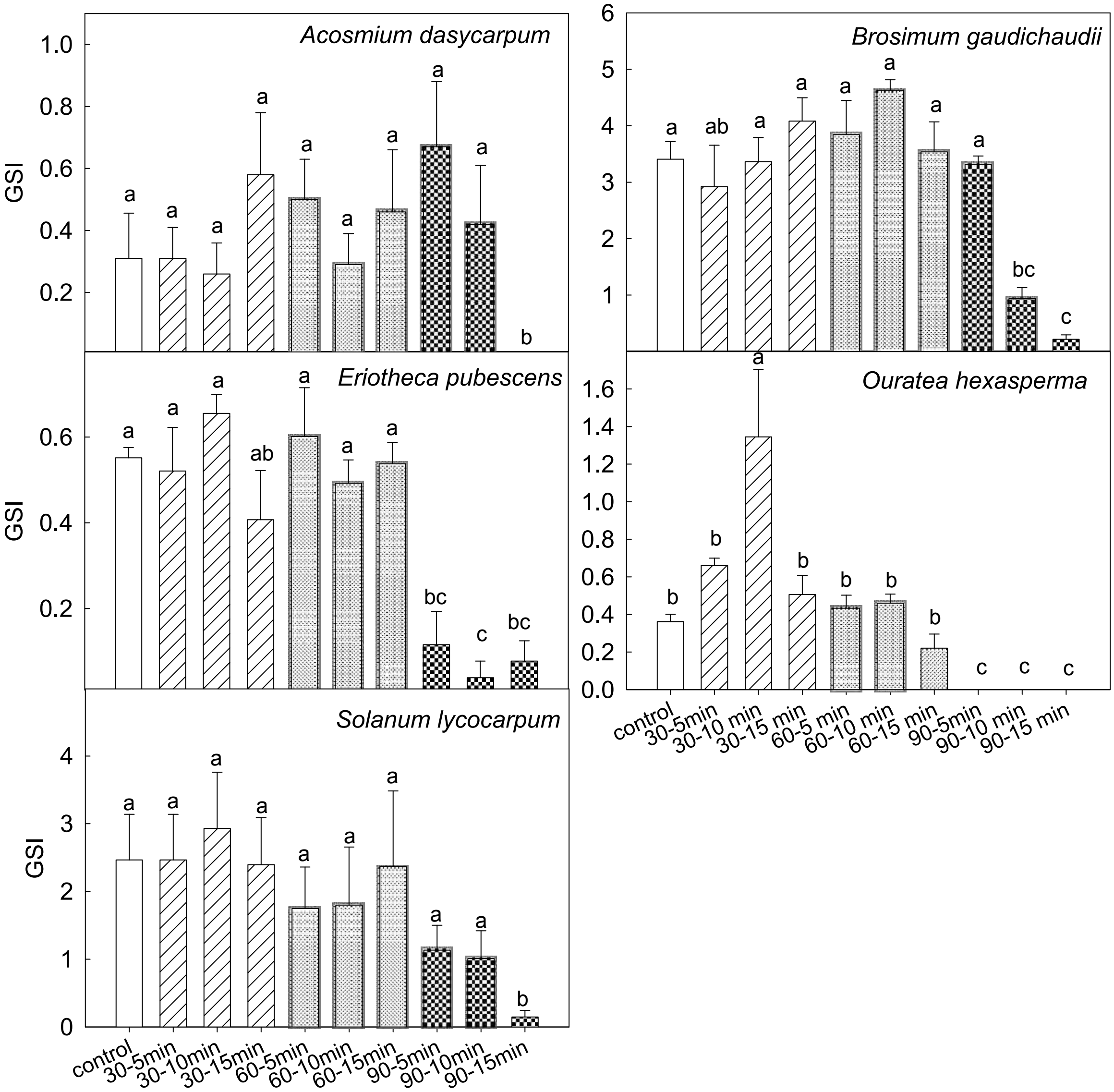


Figure 5.6. Germination speed index (GSI) of seeds of five woody species under nine heat-shock treatments (30, 60 and 90°C for 5, 10, and 15 minutes each) plus control (seeds not exposed to heat-shocks) 60 days after seed sowing. Bars are means of five replicates of 10 seeds each + SE; bars topped with the same letter do not differ significantly ($p < 0.05$) among treatments according to Tukey HSD post-hoc test.

Chapter 6

Conclusions

In this dissertation research I evaluated seed dynamics (seed rain, soil seed banks, and seed characteristics) and seedling establishment of woody species in three major cerrado vegetation types that differ in tree canopy cover and tree height. These vegetation types are located along topographic gradients of approximately 30 m in elevation and 1000 m in length. They comprised closed savannas (cerrado denso) with a nearly continuous canopy cover in the uppermost portions, open savannas (campo sujo) with scattered short trees in the low portions of the topographic gradients, and cerrado sensu stricto with intermediate canopy cover at intermediate topographic positions. The herbaceous-grass layer follows the opposite trend to woody species cover with higher abundance of herbaceous plants in open than in closed savannas. To find out if cerrado vegetation types are limited in seeds of woody species, in Chapter 2 of my dissertation, I studied spatial and temporal variations of the seed rain in three different vegetation types (closed, intermediate and open savannas). I assessed seed rain with 108 seed traps located in the field (36 per vegetation type). Using the seed rain data, I determined the degree of seed limitation of 23 woody species in each vegetation type. In the field, I also conducted experiments to determine the degree of seed predation and removal of 17 common cerrado woody species in each vegetation type. Finally, in the laboratory, I determined seed viability loss in dry-storage conditions of 12 of these common cerrado woody species. The results of Chapter 2 indicate that in the three vegetation types, the temporal variation in the number of species (herbaceous and woody) in the seed rain, particularly that of species that have wind-dispersed seeds, parallels the seasonal variation in precipitation. In addition, dispersal modes of seeds in intermediate and open savannas were highly consistent with dispersal syndromes of the local vegetation, with most animal-dispersed seeds and species in intermediate savannas, and most wind-dispersed seeds and species in open savannas. The results of Chapter 2 also indicate that the three vegetation types are limited in seeds of woody species because of low seed numbers and seeds poorly dispersed among sites. Seeds of woody species, however, are even more limited in open than in closed savannas. The results of field experiments indicate that more than 60% of seeds of 17 common cerrado species are lost by predation and removal within a period of 30-45 days, and that 66% of 12 of these species lose their seed viability in less than 12 months. Therefore, these post-dispersal events further increase seed limitation of woody species among vegetation types by quickly decreasing seed availability. Overall, the results of Chapter 2 indicate that potential recruitment of woody species along cerrado vegetation types is highly constrained by low seed supply and post-dispersal events of predation, removal, and rapid loss of viability.

Because soil seed banks may constitute an alternative source of propagules, in Chapter 3 I determined density and species composition of seeds found in the soil (soil seed bank) from each vegetation type, collected during four consecutive periods which included two dry and two wet seasons, over a two-year period. I used the percentage of species re-occurring in the soil throughout all collection periods as an indicator of the degree of soil seed bank persistence. In Chapter 3, I also assessed kind of dormancy (or lack thereof), moisture content, and longevity of seeds of 14 common cerrado woody species which differ in their dispersal season. Finally, I examined similarity patterns in woody species composition among soil seed bank, seed rain, established seedlings, and standing vegetation in the vegetation types mentioned above.

The results of Chapter 3 show that soil seed banks from closed, intermediate, and open savannas are mostly composed of herbaceous species with very few woody species. As a result, the soil seed banks of the three vegetation types do not reflect well the standing woody species composition. With the exception of two woody species that were present during all soil collection periods (*Baccharis salzmannii* and *Miconia albicans*), seeds of most cerrado woody species were absent in the soil during the collection periods. The results described in Chapter 2 regarding high seed predation, high and fast seed removal, and rapid loss of seed viability can help explain the absence of seeds of most woody species from the soil. On the other hand, the low degree of similarity (8%) between the woody species composition of the soil seed bank and the woody species composition of the standing vegetation suggests that few woody species are recruited from the soil seed bank. This low recruitment also is supported by the low similarity (12%) between the composition of woody species of the soil seed bank and the composition of established woody seedlings. The results of Chapter 3 however, show that the vegetation and the seed rain were moderately similar (52%) to one another in their species composition. Such similarity suggests that short distance dispersal is an important source of seeds and potential recruits in all three vegetation types.

The combined results of Chapters 2 and 3 show that because seed rain exhibited a strong seasonal variation with most seeds dispersed during the late dry season and early wet season, seed dispersal appears to be an important mechanism controlling timing of germination of several cerrado species. That seed dispersal synchronizes with the onset of the rainy season ensures that germination occurs when environmental conditions are suitable for seedling establishment. That seeds dispersed during the dry season had on average lower moisture content, slightly higher longevity, and a higher percentage of dormant seeds than those dispersed during the wet season, indicates that in addition to seed dispersal, dormancy also is involved in controlling timing of germination of several cerrado woody species. Seed germination at the onset of the wet season, therefore, would highly increase the chances of seedling survival during the rainy season when short-lived, non-dormant seeds are mostly dispersed.

In Chapter 4, I tested the hypothesis that seedling establishment of woody species differs among cerrado vegetation types, even in the absence of major disturbances such as fire. To test this hypothesis I evaluated density and survival of an initial seedling assemblage and of three consecutive cohorts of recruits of woody species within 216 permanent plots of $1m^2$ located in closed, intermediate and open savannas (72 per vegetation type) that have been protected from fire for at least 30 years. I quantified seedling limitation for 16 woody species as the proportion of the plots where seedlings of a particular species were not found. I also quantified light level, light quality, litter cover, soil moisture, and soil nutrient concentrations in each savanna type and performed manipulative experiments in a greenhouse to evaluate the effects of light level and litter cover on seedling emergence of 9 common cerrado woody species. The results of Chapter 4 indicate that seedling establishment differs among cerrado vegetation types: richness and density of woody seedlings was higher in closed (cerrado denso) than in open savannas (campo sujo). The difference in density was the result of high survival and recruitment in closed savannas which exhibit a relatively high canopy cover. Thus, canopy cover seems to facilitate seedling establishment by reducing stressful conditions enhanced by water deficit, irradiance, and competition with grasses. These are conditions commonly found in open savannas and could explain why recruitment of woody seedlings in this savanna type does not significantly depend on the density of pre-established seedlings. Litter cover, light quality, and light availability differed significantly from cerrado denso to campo sujo. Species responded differently to those environments under experimental conditions. High litter cover and shade significantly reduced seedling emergence of 4 out of the 9 woody species studied. In the field, however, rapid litter decomposition at the beginning of the wet season could reduce substantially litter cover thickness on the soil surface (Villalobos, unpublished results) thus favoring establishment of high litter cover-sensitive species.

The combined results of Chapters 2 and 4 suggest that in addition to seed limitation, seedling establishment limitation constrains recruitment of woody species in the cerrado. In fact, I found high seedling limitation for most of the 16 woody species in all cohorts in each savanna type, and mean limitation values were higher in open savannas than in closed savannas. Overall, the results of Chapter 4 suggest that seedling establishment and dynamics of woody species are consistent with the pattern of canopy cover variation across the cerrado landscape and that spatial and temporal heterogeneity of environmental conditions contribute to the differential establishment of seedlings among savanna types of central Brazil.

Because fire is a major disturbance factor in tropical savannas, in Chapter 5, I evaluated the effect of fire on recruitment of seedlings and vegetative reproduction of woody and herbaceous cerrado species. In the field, I quantified richness and density of seedlings and vegetative sprouts (root suckers) in fire-protected savannas and burned savannas. Burned savannas comprised accidentally burned savannas differing in tree canopy cover and a frequently burned savanna with intermediate tree canopy cover. In fire-prone ecosystems, variations in soil heating during the passage of fires can affect seedling recruitment because seeds have different tolerances to heating and different requirements for germination. Therefore, in this chapter I also evaluated the effects of pulses of heat (heat-shocks) on seed germination of five common cerrado tree species under experimentally controlled conditions. The results of Chapter 5 indicate that seedling establishment of woody and herbaceous cerrado species is lower in accidentally burned savannas and in the frequently burned savanna than in fire-protected savannas suggesting that fire highly constrains establishment of seedlings, probably by reducing seed germination of fire-sensitive species. In accidentally burned savannas and the frequently burned savannas, richness and density of vegetative sprouts were significantly higher than richness and density of seedlings suggesting that sprouting was the major means of regeneration for both woody and herbaceous species. The results of this chapter also show that fire can critically affect patterns of species composition across cerrado vegetation types. Species composition of woody and herbaceous seedlings differed significantly among fire-protected savannas, accidentally burned savannas and the frequently burned savanna, with some species restricted to either fire-protected or burned savannas. I found that seeds of five common cerrado woody species tolerated temperatures of up to 60°C for 15 minutes but did not depend on heat pulses for their germination. Heat-exposure of 90°C for longer than 10 minutes however, significantly reduced seed germination in all the studied species. These results suggest that during fires, when temperatures above soil surface can rise to 840°C, seeds near or above the soil surface are less likely to tolerate short-term soil heating than seeds buried in the soil, where temperatures of 47, 33 and 25°C have been reported at soil depths of 1, 2, and 5 cm, respectively. The results of Chapter 5 show that permanent or long-term shifts in species composition are expected under the present fire regimes in the cerrado because fire reduces post-burn seedling establishment of woody and herbaceous species, increases vegetative reproduction of some species, and differently affects seed germination of woody species.

Overall, the results of my dissertation research show that cerrado vegetation types are limited in seeds and seedlings of woody species, but these limitations are higher in open than in closed savannas. Most woody species do not form persistent soil seed banks along cerrado vegetation types because their seeds are short-lived, predated and quickly removed from the ground. In addition, dispersal season and dormancy appear to control timing of germination at the onset of the rainy season to ensure survival of seedlings in the field. In the absence of fire, establishment of woody seedlings is higher in closed than in open savannas because canopy cover mitigates the stressful environmental conditions of open savannas and thus facilitates establishment of woody seedlings. As a result, closed savannas are likely to maintain higher density of woody elements over time than open savannas. In burned savannas, however, species composition of woody and herbaceous seedlings is significantly affected because establishment of seedlings of woody and herbaceous species decreases, but vegetative reproduction increases.

The results of this study indicate that demographic bottlenecks at the early stages of the life-history of woody species may contribute to structuring cerrado vegetation types, and therefore, these results provide evidence that supports some aspects of demographic-based models for explaining savanna dynamics. Savanna dynamics have long been interpreted in the context of competition based-models, and only recently in the context of demographic-based models. Demographic-based models argue that trees and grasses persist in savannas because climatic variability and/or disturbances such as fire and grazing limit tree seed germination, seedling establishment, and /or transition to mature size classes (Sankaran et al., 2004). Competition based models, on the contrary, emphasize the fundamental role of competitive interactions in fostering the coexistence between trees and grasses in savannas. They are based on classic niche-separation mechanisms of coexistence and invoke differences in the resource-acquisition potential of trees and grasses as the fundamental process structuring savanna communities (Walker et al., 1981; van Langevelde et al., 2003). This paradigm also may explain in part some of the patterns of cerrado vegetation distribution along gradients in central Brazil. Future models should combine differences in resource acquisition as well as demographic dynamics of woody and herbaceous species to obtain comprehensive description of Neotropical ecosystems.

My study suggest that interactions of early stages with environmental and disturbance factors appear to play more significant role in maintaining woody cover variations along cerrado topographic gradients, than previously recognized. Future empirical studies that directly measure competitive and demographic effects in different savannas are highly needed to elucidate the mechanisms that control savanna structure and function. Simultaneous measurements of the demographic structure of tree communities could help identify bottlenecks and the particular stage where they occur.

Combining these data with water table dynamics, soil nutrient concentrations, soil moisture, and fire history would allow evaluation of the effects of relative competitive and demographic processes in savanna ecosystems.

References

Allen, S.E. 1989. Chemical Analysis of Ecological Materials. 2ndedition. Blackwell Scientific Publications, Oxford.

Alonso, A.A and S. R. Machado. 2007. Morphological and developmental investigations of the underground system of *Erythroxylum* species from Brazilian cerrado. Australian Journal of Botany 55: 749–758.

Amiaud, B and B. Touzard. 2004. The relationships between soil seed bank, aboveground vegetation and disturbances in old embanked marshlands of western France. Flora 199: 25-35.

Andrade, L.A Z. 2002. Impacto do fogo no banco de sementes de cerrado sensu stricto. Ph.D. Thesis. Universidade de Brasilía. Instituto de Ciencias Biologicas. Departamento de Ecologia. Brasilía, D.F. Brasil.

Asquith, N.M., S.J Wright and M.J Clauss. 1997. Does mammal community composition control recruitment in neotropical forest? Evidence from Panama. Ecology 87:941-946.

Baeten,L., H. Jacquemyn., H.V Calster., E.V Beek., R.Devlaeminck., K.Verheyen and M.Hermy. 2009. Low recruitment across life stages partly accounts for the slow colonization of forest herbs. Journal of Ecology 97:109-117.

Bansal, R.P., P.R. Bhati and D.N. Sen. 1980. Differential specificity in water imbibition of Indian arid zone seeds. Biologica Plantarum 22:327-331.

Barbosa, L. M., J. M Barbosa, C. Bendinelli., L. M Asperti,. E. F Belasque and E. A. Batista. 1993. Efeitos de geadas em mudas de especies árboreas de mata ciliar utilizadas em ensayos de campo. Acta Botânica Brasílica **7**: 95-105.

Barloto,C., D. Bonal and D.E Goldberg. 2006. Differential seedling growth response to soil resource availability among nine neotropical tree species. Journal of Tropical Ecology 22: 487-497.

Baskin, C.C and J.M Baskin. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego.666 pp.

Baskin, J.M and C.C Baskin. 2004. A classification system for seed dormancy. Seed Science and Research 14:1-16.

Baskin, C.C and J.M Baskin. 2005. Seed dormancy in trees of climax tropical vegetation types. Tropical Ecology 46:17-28.

Batalha, M.A, S.Aragaki and W. Mantovani. 1997. Variações fenológias das espécies do cerrado em Emas (Pirassununga, SP). Acta Botanica Brasilica 11:61-78.

Batalha, M.A and W. Mantovani. 2000. Reproductive phenological patterns of cerrado plant species at the Pé-de Gigante Reserve (Santa Rita do Passa Quatro, SP, Brazil): a comparison between herbaceous and the woody floras. Revista Brasileira de Biologia 60:129-145.

Batalha, M.A and F.R. Martins. 2004. Reproductive phenology of the cerrado plant community in Emas National Park (central Brazil).Australian Journal of Botany 52:149-161.

Batalla, D., B.C Kruk and L. Benech-Arnold. 2000. Very early detection of canopy presence by seeds through perception of subtle modifications in red:far red signals. Functional Ecology 14:195-202.

Bell, T.L., J.S Pate and K.W Dixon. 1996. Relationships between fire response, morphology, root anatomy and starch distribution in south-west Australian Epacridaceae. Annals of Botany 77:357-364.

Blakesley, D., S. Elliot., C.Kuarak., P.navakitbumrung., S. Zangkum and V. Anusarnsunthorn. 2002. Propagating framework tree species to restore seasonally dry tropical forest: Implications of seasonal seed dispersal and dormancy. Forest Ecology and Management 164:31-38.

Bond, W. J and B.W. van Wilgen. 1996. Fire and Plants. Chapman and Hall. 263pp.

Bond, W. J and J.J Midgley. 2001. Ecology of sprouting in woody plants: the persistence niche. TRENDS in Ecology & Evolution 16:45-51.

Bond, W.J. 2008. What limits trees in C4 grasslands and savannas? Annual Review Ecology and Systematics 39:641-659.

Breen, A.N and J.H Richards. 2008. Irrigation and fertilization effects on seed number, size, germination and seedling growth: Implications for desert shrub establishment Oecologia 157:13–19.

Campbell, M.L, and P.J. Clarke. 2006. Seed dynamics of resprouting shrubs in grassy woodlands: Seed rain, predators and seed loss constrain recruitment potential. Austral Ecology 31:1016-1026.

Castro, E.A and J.B. Kauffman. 1998. Ecosystem structure in the Brazilian cerrado: a vegetation gradient of aboveground biomass, root mass and consumption by fire. Journal of Tropical Ecology 14:263-283.

Chaideftou, E., Thanos, C.A., Bergmeier, E., Kallimanis, A and P. Dimopoulos. 2009. Seed bank composition and above-ground vegetation in response to grazing in sub-Mediterranean oak forests (NW Greece). Plant Ecology 201: 255-265.

Chang. E.R., R. L. Jefferies and T. Carleton. 2001. Relationship between vegetation and soil seed banks in an arctic coastal marsh. Journal of Ecology 89: 367-384.

Cheson, P.L. 1986. Environmental variation and the coexistence of species. *In* J.Diamond and T.Case [eds.] Community Ecology. Harper & Row, New York.

Christianini,A.V., A.J. Mayhé-Nunes and P.S. Oliveira. 2007. The role of ants in the removal of non-myermecochorous diaspores and seed germination in a neotropical savanna. Journal of Tropical Ecology 23:343-351.

Cirne, P. Da Silva. 2002. Efeitos do fogo na regeneracão de *Kielmeyera coriaceae* (Spr.) Mart. (Guttiferae) em áreas de cerrado sensu stricto: Mecanismos de sobrevivência e época de queima. Ph.D. Thesis. Universidade de Brasília, Brasília, D.F, Brazil.

Clark, J.S., E. Macklin, and L. Wood. 1998. Stages and spatial scales of recruitment limitation in southern Appalachian forests. Ecological Monographs 68:213-235.

Clark, C.J., J.R. Poulsen, D.J. Levey, and C.W. Osenberg. 2007. Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. The American Naturalist 170: 129-142.

Clarke, K.R and R.M Warwick. 2001.Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. PRIMER-E Ltd, UK.

Costa, A.N., H.L Vascocelos., E.H.m Vieira-Neto and E.M Bruna. 2008. Do herbivores exert top-down effects in Neotropical savannas? Estimates of biomass consumption by leaf-cutter ants. Journal of Vegetation Science 19:849-854.

Coutinho, L.M. 1990. Fire in the ecology of the Brazilian cerrado. *In* J.G. Goldammer [ed.] Fire in the Tropical Biota, Ecosystem Processes and Global Challenges pp:82-105. Springer-Verlag, Berlin.

Crosti, R., P.G Ladd., K.W. Dixon and B. Piotto. 2006. Post-fire germination: The effect of smoke on seeds of selected species from the central Mediterranean basin. Forest Ecology and Management 221:306-312.

Dalling, J.W., M.D Swaine and N.C Garwood. 1997. Soil seed bank community dynamics in seasonally moist lowland tropical forest, Panama. Journal of Tropical Ecology 13:659-680.

Dalling, J.W and S. P Hubbell. 2002. Seed size, growth rate and gap microsite conditions as determinants of recruitment success for pioneer species. Journal of Ecology 90:557-568.

Dalling, J.W. 2004. The fate of seed banks: Factors influencing seed survival for light-demanding species in moist tropical forests. *In* Forget, Pierre-Michel [eds.] Seed Fate: Predation, Dispersal and Seedling Establishment pp. 31-44. CAB International, Wallingford.

Deka, R.N., M.Wairiu., P.M Mtakwa., C.E Mullins., E.M Veenendaal and J.Townend. 1995. Use and accuracy of the filter-paper technique for measurement of soil matric potential. European Journal of Soil Science 46:233-238.

Denham, A.J. 2008. Seed predation limits post-fire recruitment in the waratah (*Telopea speciosissima*). Plant Ecology 199:9-19.

De Ronde, C., J.G. Goldammer., D.D Wade and R.V. Soares. 1990. Prescribed fire in industrial pine plantations. *In* J.G. Goldammer [ed.] Fire in the Tropical Biota, Ecosystem Processes and Global Challenges pp: 216-272. Springer-Verlag, Berlin.

Deveny, A.J and A.R Fox. 2006. Indirect interactions between browsers and seed predators affect the seed bank dynamics of a chaparral shrub. Oecologia 150:69-77.

Dixon., K.W., S. Roche and J.S Pate. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. Oecologia 101:185-192.

Drake, D. R. 1998. Relationships among the seed rain, seed bank and vegetation of a Hawaiian forest. Journal of Vegetation Science 9:103-112.

Durigan, G., H.F.L Filho and R.R Rodrigues. 1994. Phytosociology and structure of frequently burnt cerrado vegetation in SE-Brazil. Flora 189:153-160.

Eiten,G. 2001. Vegetação Natural do Distrito Deferal. Brasília. Editora UnB, Edição SEBRAE, 162 pp.

Eriksson, O and J. Ehrlén. 1992. Seed and microsite limitation of recruitment in plant populations. Oecologia 91:360-364.

Espinar, J.L., K. Thompson and L.V Garcia. 2005. Timing of seed dispersal generates a bimodal seed bank depth distribution. American Journal of Botany 92: 1759-1763.

Faria, P.D.I. 2004. Efeitos da predação, sazonalidade climâtica e tipo de habitat no estabelecimento e desenvolvimento das lenhosas *Kielmeyra coriacea* (Spreng) Mart. e *Qualea grandiflora* Mart. Tesis de Maestrado. Universidade de Brasília. Brasília, DF. Brazil.

Felfilli, J.M., L.F Hilgbert., A.C Franco., J.C Sousa-Silva., A.V Resende, and M.V.P Nogueira. 1999. Comportamiento de plántulas de *Sclerolobium paniculatum* Vog Var. Rubiginosum (Tul) Benth. sob diferentes níveis de sombreamento em vivero. Revista Brasileira de Botânica 22: 297-301.

Felfilli, J.M., A.V. Rezende., M.C. Da Silva Júnior and M.A. Silva. 2000. Changes in the floristic composition of cerrado sensu stricto in Brazil over a nine-year period. Journal of Tropical Ecology 16:579-590.

Felipe,G.M and J.C.S. Silva. 1984. Estudo da germinação em espécies do cerrado. Revista Brasileira de Botânica. São Paulo 7: 157-163.

Fenner, M. 1985. Seed Ecology. London. Chapman and Hall. 151pp.

Fenner, M., and K. Thompson. 2005. The Ecology of Seeds. Cambridge University Press. United Kingdom. 250 pp.

Flores, S and N. Dezzeo. 2005. Variaciones temporales en cantidad de semillas en el suelo y en lluvia de semillas en un gradiente bosque-sabana en la gran sabana, Venezuela. Interciencia 30:39-43.

Fornara, D.A and J.W Dalling. 2005. Seed bank dynamics in five Panamanian forests. Journal of Tropical Ecology 21:223-226.

Foster, B.L., and D. Tilman. 2003. Seed limitation and the regulation of community structure in oak savanna grassland. Journal of Ecology 91: 999-1007.

Fox, G.A. 2001. Failure-time analysis: Studying times to events and rates at which events occur. *In* S. M. Scheiner and J. Gerevitch [eds.] Design and Analysis of Ecological Experiments, 235-266. Oxford University Press, New York, New York, USA.

Franco, A.C. 2002. Ecophysiology of woody plants. *In* P.S. and R.J. Marquis [eds.] The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna pp. 178-197.Columbia University Press. NY.

Furley P.A and J.A. Ratter. 1988. Soil resources and plant communities of the central Brazilian cerrado and their development. Journal of Biogeography 15:97-108.

Furley, P.A. 1999. The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. Global Ecology and Biogeography 8:223-241.

Gardner, T.A. 2006. Tree-grass coexistence in the Brazilian cerrado: demographic consequences of environmental instability. Journal of Biogeography 33:448-463.

Garwood, N.C. 1983. Seed germination in a seasonal tropical forest in Panama: a community study. Ecological Monographs 53:159-181.

Garwood, N.C. 1989.Tropical soil seed banks: a review. *In* Leck, M.A., V.T Parker and R. L Simpson [eds.] Ecology of Soil Seed Banks. Academic Press, San Diego.

Gignoux, J., J. Clobert and J.C. Menaut. 1997. Alternative fire resistance strategies in savanna trees. Oecologia 110:576-583.

Gignoux, J., G.Lahorearu., R.Julliard and S. Barot. 2009. Establishment and early persistence of tree seedlings in annually burned savanna. Journal of Ecology 97:484-495.

Goldstein,G., F.C. Meinzer., S. Bucci., F.G. Scholz., A.C. Franco and W. A. Hoffmann. 2008. Water economy of Neotropical savanna trees: six paradigms revisited. Tree physiology 28:395-404.

Gom, L.A and S.B. Rood. 1999. Fire induces asexual sprouting of riparian cottonwoods. Canadian Journal of Botany 77: 1604-1616.

Goodland, R 1971. A physiognomic analysis of the “cerrado” vegetation of central Brazil. Journal of Ecology 59:411-419.

Goodland, R and R. Pollard. 1973. The Brazilian cerrado vegetation: a fertility gradient. Journal of Ecology 61:219-224.

Gottsberger,G and I. Silberbauer- Gottsberger. 2006. Life in the Cerrado a South Tropical Seasonal Ecosystem. Vol I. Origin, Structure, Dynamics and Plant Use. Ulm: Reta Verlag. 277 pp.

Gottsberger,G and I. Silberbauer- Gottsberger. 2006. Life in the Cerrado a South Tropical Seasonal Ecosystem. Vol II. Pollination and Seed Dispersal. Ulm: Reta Verlag. 383 pp.

Gouveia, G.P and J.M Felfili. 1998. Fenologia das comunidades de cerrado e mata de galleria no Brasil central. Revista Árvore 22:443-450.

Grombone-Guaratini, M.T and R.Ribeiro-Rodrigues. 2002. Seed bank and seed rain in a seasonal semi-deciduous forest in south-eastern Brazil. Journal of Tropical Ecology 18:759-774.

Hagenah, N., H. Munkert., K. Gerhardt and H. Olff. 2009. Interacting effects of grass height and herbivores on the establishment of an encroaching savanna shrub. Plant Ecology 201: 553-566.

Hall, J.B and M.D. Swaine. 1980. Seed stocks in Ghanaian forest soils. Biotropica 12:256-263.

Haridasan, M. 2008. Nutritional adaptations of native plants of the cerrado biome in acid soils. Brazilian Journal of Plant Physiology 20:183-195.

Harper, J.L. 1977. Population Biology of Plants. Academic Press. 892 pp.

Heringer, E.P. 1971. Propagação e sucessão de espécies arbóreas do cerrado em função do fogo, do cupim, da capina e do aldrim (inseticida). *In* III Simpósio sobre o cerrado pp167-179.

Higgins, S.I., W.J. Bond and W.S.W Trollope. 2000. Fire, resprouting and variability: A recipe for grass-tree coexistence in savanna. Journal of Ecology 88:213-229.

Higgins, S.I., W.J. Bond., E.C. February., A.Bronn., D.I.W Euston-Brown., B.Enslin., N.Govender., L.Rademan., S.O'Regan., A.L.F. Potgieter., S.Scheiter., R.Sowry., L.T Trollope and W.S.W Trollope. 2007. Effects of four decades of fire manipulation on woody vegetation structure in savanna. Ecology 88:1119-1125.

Hoffmann, W.A. 1996. The effects of fire and cover on seedling establishment in a neotropical savanna. Journal of Ecology 84:383-393.

Hoffmann, W.A. 1998. Post-burn reproduction of woody plants in a neotropical savanna: the relative importance of sexual and vegetative reproduction. Journal of Applied Ecology 35:422-433.

Hoffmann, W.A. 2000. Post-establishment seedling success in the Brazilian cerrado: A comparison of savanna and forest species. Biotropica 32: 62-69.

Hoffmann, W.A. 2002. Direct and indirect effects of fire on radial growth of cerrado savanna trees. Journal of Tropical Ecology 18:137-142.

Hoffmann, W.A and A.G Moreira. 2002. The role of fire in population dynamics of woody plants. *In* P.S. and R.J. Marquis [eds.] The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna pp. 150-177 Columbia University Press. NY.

Hoffmann, W.A and A.C Franco. 2003. Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts. Journal of Ecology 91:475-484.

Hoffmann, W.A and O.T.Solbrig. 2003. The role of topkill in the differential response of savanna woody species to fire. Forest Ecology and Management 180:273-286.

Hoffman, W.A., E.R da Silva., G.C Machado., S.J Bucci., F.G Scholz., G. Goldstein and F.C Meinzer. 2005. Seasonal leaf dynamics across a tree density gradient in a Brazilian savanna. 145:307-316.

Hoffmann,W.A and M. Haridasan. 2008. The invasive grass, *Melinis minutiflora*, inhibits tree regeneration in an Neotropical savanna. Austral Ecology 33:29-36.

Hopfensperger,K.N. 2007. A review of similarity between seed bank and standing vegetation across ecosystems. Oikos 116:1438-1448.

Hovstad, K.A and M.Ohlson. 2008. Physical and chemical effects of litter on plant establishment in semi-natural grasslands. Plant Ecology 196:251-260.

Hubbell, S.P., R.B Foster., S.T Obrien. 1999. Light-gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. Science 238:554-557.

Hui, L and W. Kepin. 2006. Soil seed bank and aboveground vegetation within hillslope vegetation restoration sites in Jinshajing hot-dry river valley. Acta Ecologica Sinica 26:2432-2442.

Hurtt, G.C and S.W Pacala. 1995. The consequences of recruitment limitation: Reconciling chance, history and competitive differences between plants. Journal of Theoretical Biology 176:1-12.

Ikeda, F.S., D. Mitja., L. Vilela and J.C Sousa-Silva. 2008. Banco de sementes em cerrado sensu stricto sob queimada e sistemas de cultivo. Pesquisa Agropecuaria Brasileira, Brasília 43:667-673.

Jackson, P.C., F.C. Meinzer., M.Bustamante., G.Goldstein., A. Franco., P.W. Rundel., L. Caldas., E. Ingler and F. Causin. 1999. Partitioning of soil water among tree species in a Brazilian cerrado ecosystem. Tree Physiology 19: 717-724.

Jeltsch, F.,S.J Milton., W.R.J Dean., N.Van Rooyen and K.A Moloney. 1998. Modelling the impact of small-scale heterogeneities on tree-grass coexistence in semi-arid savannas. Journal of Ecology 86:780-793.

Joly, C.A. 1996. The role of oxygen diffusion to the root system on the flooding tolerance of tropical trees. Revista Brasileira de Biologia 56: 375-382.

Kanegae, M.F., V. Da S. Braz and A.C. Franco. 2000. Efeitos da seca sazonal e disponibilidade de luz na sobrevivência e crescimento de *Bowdichia virgilioides* em duas fitofisionomias típicas dos cerrados do Brasil central. Revista Brasileira de Botânica, São Paulo 23:459-468.

Keeley, J.E. 1992. Recruitment of seedlings and vegetative sprouts in unburned chaparral. Ecology 73:1194-1208.

Keeley J.E and C.J. Fotheringham. 1998. Smoke-induced seed germination in California chaparral. Ecology 79: 2320-2336.

Kollmann, J and D. Goetze. 1998. Notes on seed traps in terrestrial plant communities. Flora 193:31-40.

Krebs, C.J. 1999. Ecological Methodology. Addison-Wesley Educational Publishers, Inc Menlo Park, California, USA.

Labouriau, L.G., I.F Valio., M.L.S Labouriau and W. Handro. 1963. Nota sobre a germinação de sementes de plantas de cerrados em condições naturais. Revista Brasileira de Biologia 23: 227-237.

Lacey, C.J and R.D Johnston. 1990. Woody clumps and clumpwoods. Australian Journal of Botany 38:299-334.

Lambers, H., F.S Chapin, and T.L Pons. 1998. Plant Physiological Ecology. Springer-Verlag. New York.

Langevelde, V. F., van De Vijer, C.A.D.M., Kumar,L., van De Koppel, J., De Ridder, N., Van Andel, J., Skidmore, A.K., Hearne, J.W., Stroosnijder, L., Bond, W.J., Prins, H.H.T and M. Rietkerk. 2003. Effects of fire and herbivory on the stability of savanna ecosystems. Ecology 84: 337-350.

Leal, I.R and P.S. Oliveira. 1998. Interactions between fungus-growing ants (Attini), fruits and seeds in cerrado vegetation in southeast Brazil. Biotropica 30: 170-178.

Ledru, M.P. 2002. Late quaternary history and evolution of the cerrados as revealed by palynological records. *In* P.S. and R.J. Marquis [eds.] The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna pp. 33-50. Columbia University Press. NY.

Lenza, E and C.A Klink. 2006. Comportamento fenológico de espécies lenhosas em um cerrado sentido restricto de Brasília, D.F.Revista Brasileira de Botânica 29:627-638.

Maguire, J.D. 1962. Speed of germination: Aid in selection and evaluation for seedling emergence and vigor. Crop Science 1:176-177.

Mantovani, W and F.R. Martins. 1988.Variações fenológicas das espécies do cerrado da Reserva Biologica de Mogi-Guacu, estado de São Paulo. Revista Brasileira de Botânica 3:227-237.

Marimon, B.S and J.M Felfilli. 2006. Chuva de sementes em uma floresta monodominante de *Brosimum rubescens* Taub. e em uma floresta mista adjacente no Vale do Araguaia, M.T. Brasil. Acta Botânica Brasílica 20: 423-432.

Matlaga, D and C.C. Horvitz. 2009. Growth and survival across a gap-understory gradient: contrast in performance of sexually vs. clonally produced offspring. American Journal of Botany 96:439-447.

Matos, M. R. B. 1994. Efeito do fogo sobre os regenerantes de *Blepharocalix salicifolius* H.B.K (Myrtaceae) em cerrado aberto, Brasília, D.F. Master Thesis. Universidade de Brasília, Brasil.

McIvor, J.G and C. J Gardener. 1991. Soil seed densities and emergence patterns in pastures in the seasonally dry tropics of northeastern Australia. Australian Journal of Ecology 16:159-169.

Medina, E and J.F Silva. 1990. Savannas of the northern South America: a steady state regulated by water-fire interactions on a background of low nutrient availability. Journal of Biogeography 17: 403-413.

Melo, J.T. de; J.A da Silva,. R.A.de A Torres,. C.E. dos S Silveira and L.S Caldas. 1998. Colecta, propagação e desenvolvimento inicial de espécies do cerrado. *In* S.M Sano and S.P Almeida [eds.] Cerrado: Ambiente e Flora. Planaltina pp 195-243. EMBRAPA-CPAC.

Meyer, K.M., K. Wiegand., D. Ward and A. Moustakas. 2007. SATCHMO: A spatial simulation model of growth, competition, and mortality in cycling savanna patches. Ecological Modelling 209:377-391.

Miranda A.C., H.S. Miranda., I.F. Oliveira and B.F De Souza. 1993. Soil and air temperatures during prescribed cerrado fires in central Brazil. Journal of Tropical Ecology 9: 313-320.

Miranda H.S., M. Bustamante and A.C Miranda. 2002. The fire factor. *In* P.S Oliveira and R.J. Marquis [eds.] The Cerrados of Brazil: Ecology and Natural History of a Neotropical savanna. pp. 51-68.Columbia University Press. NY.

Mistry, J and A. Berardi. 2005. Assessing fire potential in a Brazilian savanna nature reserve. Biotropica: 37:439-451.

Moles, A.T, and M. Westoby. 2002. Seed addition experiments are more likely to increase recruitment in larger-seeded species. Oikos 99:241-248.

Molofsky, J and C.K. Augspurger. 1992. The effect of leaf litter on early seedling establishment in an tropical forest. Ecology 73:68-77.

Moreira, A.G. 2000. Effects of fire protection on savanna structure in central Brazil. Journal of Biogeography 27 (4), 1021-1029.

Moreira, A.G and C.A. Klink. 2000. Biomass allocation and growth of tree seedlings from two contrasting Brazilian savannas. Ecotropicos 13 (1): 43-51.

Muller-Landau, H.C., S.J. Wright, O.Calderón, S.P. Hubbell, and R.B. Foster. 2002. Assessing recruitment limitation: Concepts, methods and case-studies from a tropical forest. *In* D.J Levey, W.R. Silva and M. Galetti [eds.] Seed Dispersal and Frugivory: Ecology, Evolution and Conservation pp 35-53. CAB International, Wallingford.

Munhoz, C.B.R and J.M Felfili. 2007. Reproductive phenology of an herbaceous-subshrub layer of a savannah (campo sujo) in the cerrado Biosphere Reserve I, Brazil. Brazilian Journal of Biology 67:299-307.

Murray, K.G and J.M.Garcia. 2002. Contributions of seed dispersal and demography to recruitment limitation in a Costa Rican cloud forest *In* D.J Levey, W.R. Silva and M. Galetti [eds.] Seed Dispersal and Frugivory: Ecology, Evolution and Conservation pp 323-338. CAB International, Wallingford.

Nardoto, G.B., Souza, M.P & A.C Franco. 1998. Estabelecimento e padrões sazonais de produtividade de *Kielmeyra coriacea* (Spr) Mart. Nos cerrados do Planalto central: efeitos do estresse hidrico e sombreamento. Revista Brasileira de Botânica 21:313-319.

Nathan, R and H.C. Muller-Landau. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. TREE 15:278-285.

Nefabas, L.L and J. Gambiza. 2007. Fire-tolerance mechanisms of common woody plant species in a semiarid savanna in south-western Zimbabwe. African Journal of Ecology 45:550-556.

Oliveira, P.E and A Moreira. 1992. Anemocoria em espécies de cerrado e mata de galeria de Brasília, Distrito Federal. Revista Brasileira de Botânica 15:163-174.

Oliveira, P.E and J.C.S Silva. 1993. Reproductive biology of two species of *Kielmeyera* (Guttiferae) in the cerrados of central Brazil. Journal of Tropical Ecology 9:67-79.

Oliveira, P.E. 1998. Fenologia e biologia reprodutiva das espécies de cerrado *In* S.M Sano and S.P Almeida [eds.] Cerrado: Ambiente e Flora. Pp 170-192. Planaltina. EMBRAPA-CPAC.

Oliveira –Filho, T and J.A Ratter. 2002. Vegetation physiognomies and woody flora of the cerrado biome. *In* Oliveira, P.S. and R.J. Marquis [eds.] The Cerrados of Brazil: Ecology and Natural History of a Neotropical savanna. pp 33-51. Columbia University Press. NY. USA.

Oliveira, P.S and R.J. Gibbs. 2002. Pollination and reproductive biology in cerrado plant communities *In* Oliveira, P.S. and R.J. Marquis [eds.] The Cerrados of Brazil: Ecology and Natural History of a Neotropical savanna. pp 329-347. Columbia University Press. NY. USA.

Overbeck G.E., S.C. Muller., V.D. Pillar and J. Pfadenhauer. 2006. No heat-stimulated germination found in herbaceous species from burned subtropical grassland. Plant Ecology 184: 237-243.

Parr, C.L., A.N. Andersen., C. Chastagnol and C. Duffaud. 2007. Savanna fires increase rates and distances of seed dispersal by ants. Oecologia 151:33-41.

Paulillo, M.T.S., G.M. Felippe and J.E. Dale. 1993. Crescimento inicial de *Qualea grandiflora*. Revista Brasileira de Botânica 16: 37-46.

Pearson, T.R.H., D.F.R. P Burslem., C.E Mullins and J.D. Dalling. 2003. Functional significance of photoblastic germination in neo-tropical pionner trees: a seed's eye view. Functional Ecology 17: 394-402.

Peco, B., M. Ortega and C. Levassor. 1998. Similarity between seed bank and vegetation in Mediterranean grassland: a predicitve model. Journal of Vegetation Science 9: 815-828.

Perez, E. M and E. T Santiago. 2001. Dinámica Estacional del Banco de Semillas en una Sabana en los Llanos Centro-Orientales de Venezuela. Biotropica 33:435-446.

Perez, E.M., M Weisz., P. Lau and L. Bulla. 2006. Granivory, seed dynamics and suitability of the seed-dish technique for granivory estimations in a neotropical savanna. Journal of Tropical Ecology 22:255-265.

Pinheiro, J.C and D.M. Bates. 2000. Mixed-effects models in S and S-plus. Springer-Verglag. New York, Inc.

Pivello, A.R and L.M Coutinho. 1996. A qualitative successional model to assist in the management of Brazilian cerrados. Forest Ecology and Management. 87: 127-138.

Portnoy, S and M.F. Wilson. 1993. Seed dispersal curves: the behavior of the tail of the distribution. Evolutionary Ecology 7:25-44.

Poulsen, J.R., C.W Osenberg., C.J Clark., D.J Levey and B.M Bolker. 2007. Plants as reef fish: Fitting the functional form of seedling recruitment. The American Naturalist 170:167-183.

Ramos, A.E. 2004. Efeito do fogo bienal e quadrienal na estructura populacional e reprodução de quatro espécies vegetais do cerrado sensu stricto. Ph.D. Thesis. Universidade de Brasília, Brasília D.F, Brazil.

Rao, M., J. Terborgh and P. Nunez. 2001. Increased herbivory in forest isolates: Implications for plant community structure and composition. Conservation Biology 15: 624-633.

Ratter, J.A., J.F Ribeiro and S.Bridgewater. 1997. The Brazilian cerrado vegetation and threats to its biodiversity. Annals of Botany 80: 223-230.

Read, T.R., S.M Bellairs., D.R Mulligan and D. Lamb. 2000. Smoke and heat effects on soil seed bank germination for re-establishment of a native forest community in New South Wales. Austral Ecology 25:48-57.

Rizzini, C.T and E. P.Heringer. 1962. Studies on the underground organs of trees and shrubs from some southern Brazilian savannas. Anais Academia Brasileira de Ciências 34:235-251.

Rizzini, C.T. 1965. Experimental studies on seedling development of cerrado woody plants. Annals of the Missouri Botanical Garden 52:410-426.

Rizzini,C.T. 1976. Influência da temperatura sobre a germinação de diásporos do cerrado. Rodriguesia 41:341-383.

Roberts, E. H. 1973. Predicting the storage life of seeds. Seed Science and Technology 1: 499-514.

Saha, S and H.F.Howe. 2003. Species composition and fire in a dry deciduous forest. Ecology 84:3118-3123.

Sankaran, M., Ratnam J and N. P Hanan. 2004. Tree-grass coexistence in savannas revisited insights from an examination of assumptions and mechanisms invoked in existing models. Ecology Letters 7:480-490.

Sarmiento, G and M. Monasterio. 1983. Life forms and phenology. *In* F. Bourlière Goldammer [ed.] Tropical Savannas Ecosystems of the World 13 pp: 79-108. Elsevier, Amsterdam.

Sarmiento, G. 1984. The Ecology of Neotropical Savannas. Harvard University Press, Cambridge, MA.

Sassaki, R.M & G.M Felippe. 1997. Soil type and early growth pattern in *Dalbergia miscolobium* Benth, a cerrado tree species. Revista Brasileira de Bôtanica 57:603-610.

Sassaki, R.M and G.M Felippe. 1999. Estabelecimento de plântulas de *Dalbergia miscolobium* Benth, uma espécie arbórea dos cerrados brasileiros. Tropical Ecology 40:33-40.

Sassaki, R.M., J. N. Rondon., L.B.P Zaidan and G.M Felippe. 1999. Number of buried seeds and seedling emergence in cerradão, cerrado and gallery forest soils at Pedregulho, Itirapina (SP), Brazil. Revista Brasileira de Botânica, São Paulo 22:147-152.

Sato, M.N. 2003. Efeito ao longo prazo de queimadas prescritas na estrutura da comunidade de lenhosas da vegetação do cerrado sensu stricto. Ph.D. thesis. Universidade de Brasília, Brasília, D.F, Brasil.

Saulei, S.M and M.D Swaine. 1988. Rain forest seed dynamics during succession at Gogol, Papua New Guinea. Journal of Ecology 76:1133-1152.

Sautu, A. 2004. Ecology, morphology, and germination physiology of tree seeds in a tropical semievergreen forest in the Panama canal watershed, with special reference to seed dormancy classes along a precipitation gradient. Master Thesis. University of Kentucky, USA.

Sautu, A., J.M Baskin., C.C Baskin., J. Deago and R. Condit. 2007.Classification and ecological relationships of seed dormancy in a seasonal moist tropical forest, Panama, central America. Seed Science Research 17:127-140.

Scarpa, F.M and I.F.M Valio. 2008. Relationship between seed size and litter effects on early seedling establishment of 15 tropical tree species. Journal of Tropical Ecology 24:569-573.

Schafer, M and P.M Kotanen. 2003. The influence of soil moisture on losses of buried seeds to fungi. Acta Oecologica. 24:255-263.

Scholes, R.J and S. R Archer. 1997. Tree-grass interactions in savannas. Annual Review of Ecology and Systematics 28:517-544.

Setterfield, S.A. 2002. Seedling establishment in an Australian tropical savanna: effects of seed supply, soil disturbance and fire. Journal of applied Ecology 39:949-959.

Silva, D.M da Silva and M.A Batalha. 2008. Soil-vegetation relationships in cerrados under different fire frequencies. Plant Soil 311:87-96.

Silva, J.F., A. Zambranoa and R. Mario. 2001. Increase in the woody component of seasonal savannas under different fire regimes in Calabozo, Venezuela. Journal of Biogeography, 28, 977-983.

Silva, J.F., M.R Fariñas., J.M Felfili and C.A Klink. 2006. Spatial heterogeneity, land use and conservation in the cerrado region of Brazil. Journal of Biogeography 33:536-548.

Silva Júnior, M.C. 2005. 100 Árvores do Cerrado: Guia de Campo. Ed.Rede de Sementes do Cerrado. 278pp.

Simpson, R.L., M. A. Leck and V. T. Parker. 1989. Seed Banks: General Concepts and Methodological Issues. *In* Leck ,M. A., V. T. Parker and R.L Simpson [eds.] Ecology of Soil Seed Banks pp 3-8. Academic Press Inc.

Souza, M.F.L. 1993. Chuva de sementes em areas de campo sujo e cerrado em Brasília, D.F. Tesis de Maestrado. UnB. Dpto de Ecologia. 142 pp.

Stevenson, P. R and I.N. Vargas. 2008. Sample size and appropriate design of fruit and seed traps in tropical forests. Journal of Tropical Ecology 24:95-105.

Swaine, M.D., W.D. Hawthorne and T.K. Orgie. 1992. The Effects of fire exclusion on savanna vegetation at Kpong, Ghana. Biotropica 24:166-172.

Terborgh, J and S. J Wright. 1994. Effects of mammalian herbivores on plant recruitment in two neotropical forests. Ecology 75:1829-1833.

Thomas, P.B., E. Charles Morris and T.D. Auld. 2007. Response surfaces for the combined effects of heat-shock and smoke on germination of 16 species forming soil seed banks in south-east Australia. Austral Ecology 32:605-616.

Thompson, K and J.P Grime. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67:893-921.

Turnbull, L.A., M.J Crawley, and M.Rees. 2000. Are plant populations seed-limited? A review of seed sowing experiments. Oikos 88: 225-238.

Tyler, C. 1995. Factors contributing to postfire seedling establishment in chaparral: direct and indirect effects of fire. Journal of Ecology 83: 1009-1020.

Uriarte, M., C. D Caham., J. Thompson., J.K Zimmerman and N. Brokaw. 2005. Seedling recruitment in a hurricane-driven tropical forest: light limitation, density-dependence and the spatial distribution of parent trees. Journal of Ecology 2005:291-304.

Valio, I.F and F.M Scarpa. 2001. Germination of seeds of tropical pioneer species under controlled and natural conditions. Revista Brasileira de Botânica. São Paulo. 24:79-84.

Vander Wall, S.B., K.M. Kuhn, and M.J. Beck. 2005. Seed removal, seed predation and secondary dispersal. Ecology 86: 801-806.

Vázquez-Yanes, C., A. Orozco-Segovia., E. Rincón., M.E Sánchez-Coronado., P.Huante., J.R Toledo and V.L Brarradas. 1990. Light beneath the litter in a tropical forest: effect on seed germination. Ecology 7:1952-1958.

Vázquez-Yanes, C and A. Orozco-Segovia. 1993. Patterns of seed longevity and germination in the tropical rainforest. Annual Review Ecology and Systematics 24:69-87.

Walk, J.L., Baskin, J.M and C.C Baskin. 1996. An ecologically and evolutionary meaningful definition of a persistent seed bank in *Solidago*. American Journal of Botany 83 (Supplement N° 6), 78-79 [Abstract]

Walker, B.H., D. Ludwig., C.S Holling and R.M Peterman. 1981. Stability of semi-arid savannah grazing systems. Journal of Ecology 69:473-498.

Wenny, D.G. 2000. Seed dispersal, seed predation, and seedling recruitment of a neotropical montane tree. Ecological Monographs 70:331-351.

Williams, P.R., R.A. Congdon., A.C. Grice and P.J Clarke. 2005. Germinable soil seed banks in a tropical savanna: seasonal dynamics and effects of fire. Austral Ecology 30: 79-90

Wills, T.J and J. Read. 2007. Soil seed bank dynamics in post-fire heathland succession in south-eastern Australia. Plant Ecology 190:1-12.

Young, K.R., J.J Ewel and B.J Brown. 1987. Seed dynamics during forest succession in Costa Rica. Vegetatio 71:157-173.

Zobel, M., M. Otsus., J. Liira., M. Moora and T. Mols. 2000. Is small-scale species richness limited by seed availability or microsite availability? Ecology 81: 3274-3282