Consequences of agroforestry management on understory vegetation and soils in Uruguay

Laura J. Six

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

Doctor of Philosophy

University of Washington

2012

Reading committee:
Jonathan D. Bakker, chair
Robert E. Bilby
Robert L. Edmonds

Program Authorized to Offer Degree:
School of Environmental and Forest Sciences

University of Washington

Abstract

Consequences of agroforestry management on understory vegetation and soils in Uruguay

Laura J. Six

Chair of the Supervisory Committee:
Assistant Professor Dr. Jonathan D. Bakker
School of Environmental and Forest Sciences

Uruguay, part of the Campos ecoregion, which has a long history of grazing. Recently, afforestation has also become a common land-use change across the Campos. As a novel ecosystem develops, the effects of these multiple disturbances are largely unknown. In this dissertation, I present results of field experiments to provide some understanding of the interactive effects of grazing and afforestation in the Campos.

First, I described vegetation change across a *Eucalyptus grandis* agroforestry management cycle by characterizing richness and composition of understory vegetation across five phases of a plantation cycle (Grassland, Young Forest, Mid-stage Forest, Old Forest, and Post-Harvest). I found that mid-stage forests were most different in richness and composition from other phases, especially Grasslands, and that sites exhibited potential for recovery after harvest, as richness increased in Old Forests and Post-Harvest sites.

Second, I characterized soil physical and chemical properties across the same *Eucalyptus grandis* cycle. I found that soils appeared largely unaffected by afforestation (instead differing primarily with sample depth), which may be a result of limitations in sample size, as well as already reduced fertility from long-term grazing.

Third, I used a manipulative approach to quantify the individual and interactive effects of grazing and afforestation on vegetation at multiple spatiotemporal scales. From species area curves, I found that small-scale richness was greater in grasslands, but a greater rate of species accumulation in forests resulted similar richness at larger scales. At the site-level, while seasonal changes in vegetation were evident in grazed areas, the removal of grazing resulted differing richness by habitat: richness decreased in grasslands with time since exclosure, but increased in forests. My results demonstrated a complex response of vegetation to disturbance, that varied by the nature of the disturbance mechanism.

Finally, I quantified pine seedling germination and establishment in plantations and adjacent grasslands to examine potential spread of this exotic species. I found that seedling density in grasslands was minimal compared to plantations, and that the mechanisms controlling encroachment differed between grazed and ungrazed areas: in ungrazed grasslands, the dense vegetation cover prevents establishment, whereas in grazed grasslands, intensive livestock grazing prevents tree establishment.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Number Page

LIST OF TABLES

Table Number Page

ACKNOWLEDGEMENTS

While specific acknowledgements are included at the end of each chapter, many people warrant acknowledgement for their involvement in this research project overall. My co-advisors, Jon Bakker and Bob Bilby, have been invaluable; they have been generous with their time, and have provided insight, advice, and encouragement on all aspects of this project, from field work to manuscript writing. I am also grateful to my other advisors, María Bemhaja, Bob Edmonds, and Josh Tewksbury for their involvement. I wish to María Bemhaja, especially, for her taxonomic assistance in Uruguay, providing understanding of grazing and grassland ecology, and coordinating with the INIA lab for soils analysis and further field and lab assistance.

Many colleagues from Weyerhaeuser helped in various ways. Steve Duke greatly helped with statistical techniques during study design and analysis of each individual project. Luciana Ingaramo, Juliana Ivanchenko, and Juan Pedro Posse all helped with the logistics of field work in Uruguay, and not only helped locate field sites and provide field assistance, but offered encouragement as well. My field seasons in Uruguay would not have been successful without two incredible field assistants, Cat Adams and Scott Batiuk. Both Cat and Scott were unbelievably hard working and tenacious, and they made long field seasons enjoyable, with their positive attitudes, humor and camaraderie while we stayed in Uruguay.

In total, I spent seven months in Uruguay over the course of two years, and I must thank one family for being the primary reason this has been an unforgettable experience. Sonia, Kike, and Luana (my Uruguayan “family”) not only made my stay in Uruguay as comfortable as possible, but also opened their lives up to my crew and me. Because of them, memories of field work are also intertwined with memories of traditional asados, live music, horse rides, laughter, and dulce de leche.

I could not have completed this dissertation without the support of my friends and family. So many people offered words of encouragement, asked questions of interest, and helped me have a life outside of my dissertation. I thank my parents, especially, for believing that I could do this, and for helping in the field by playing with foil and funnels, and taking notes in the rain.

Most importantly, I thank John. I cannot fully express my gratitude for everything he did throughout this process. He has been extremely compassionate and supportive, accommodating my need to be away for several weeks at a time during field season and diffusing my anxiety over completing this huge endeavor. Most of all, he found enjoyment in the experience, too, and helps keep Uruguay close at heart, sharing in my appreciation of morcilla and mojella, learning to cook parilla-style, and serving pisco sours whenever the mood strikes.

This dissertation is for you…for us.

Chapter 1
A general overview of agroforestry effects on understory vegetation and soils in Uruguay

Uruguay, as well as southern Brazil and northern Argentina, comprise the Campos ecoregion, one of the most extensive grasslands globally. Most (85%) of Uruguay is considered natural grassland, dominated by perennial grass and herb species (Pallares *et al.* 2005). Domestic herbivores were introduced during the sixteenth century (Paruelo *et al.* 2007), and now, the Campos supports 10.1 million cattle and 13 million sheep. Grazing has now been a long-term and continual influence on this natural grassland ecosystem (Pallares *et al.* 2005) and has been a primary determinant of understory species composition and structure (Soriano *et al.* 1991, Altesor *et al.* 1998, Paruelo *et al.* 2007).

In 1987, the government of Uruguay passed a forestry law to promote forest industry investment. Forest plantations are being established where none existed in recorded history. As a result of this law, 23% of the land in Uruguay (approximately 4 million ha) has been designated for afforestation, of which nearly 1 million ha have already been planted (Uruguay XXI 2010). The large scale of the afforestation effort in Uruguay, and across the Campos, is decreasing the historic dominance of historically grazed grasslands on the landscape (Carrere & Lohmann 1996, Geary 2001, Baldi *et al.* 2006, Dumig *et al.* 2008, Baldi & Paruelo 2008, Vega *et al.* 2009).

The Campos system is a good example of a novel ecosystem, a concept that has recently emerged (Hobbs *et al.* 2006), and describes an ecosystem that has evolved with chronic human disturbance. Although novel ecosystems exhibit conditions that differ from those that existed prior to human influence, these systems can have conservation value (Hobbs *et al.* 2006, Lindenmayer *et al.* 2008, Hobbs *et al.* 2009). However, in order to manage these altered systems in a manner that retains their conservation value, it is necessary to understand how resilient they are to new disturbance factors that may be introduced on the landscape (Seastedt *et al.* 2008). The imposition of afforestation on the Campos represents just such a new disturbance element for the Campos. In this dissertation, I explore changes caused by agroforestry (the combination of afforestation and grazing) to vegetation and soils, by presenting four different studies. These studies both contribute to our understanding of the flora of the grazed Campos grasslands, which have been studied relatively little, and provide an indication of how this vegetation may respond to large-scale afforestation.

In the first study, I characterize vegetation change across a first-rotation of *Eucalyptus grandis*, examining changes in species richness, composition, and proportional importance across five phases of agroforestry management (Grasslands, Young Forests, Mid-stage Forests, Old Forests, and Post-Harvest) (Chapter 2). I also characterized changes in soil physical and chemical characteristics across the five phases of agroforestry management, as changes in soil characteristics can fundamentally alter vegetation (Chapter 3) (Kimmins 1997, Paul *et al.* 2002, Browning *et al.* 2008). After characterizing changes in vegetation with agroforestry (afforestation and grazing), I investigate the separate and interactive effects of grazing and afforestation on understory vegetation by comparing grazed and ungrazed areas in *Pinus taeda* and grassland sites (Chapter 4). Finally, because *Eucalyptus* and *Pinus* species that are typically planted in the Campos are exotic to the region, I examined *Pinus taeda* seedling germination and establishment in plantations and adjacent grasslands, to see plantations posed a threat of encroachment into remnant grasslands.

Note to the reader: Chapters 2-5 are each intended as a separate manuscript for publication. Therefore, each chapter includes an abstract, and is intended to be a stand-alone manuscript to be later published.

Chapter 2
Understory vegetation changes over an agroforestry management cycle in Uruguay

Abstract

Disturbance may be a driver of species richness or diversity in many systems, but the interactive effects of multiple disturbances are less understood. The Campos ecoregion of South America is one of the most extensive grasslands globally, and has long been impacted by grazing. Recently, afforestation has become a common land-use change across the Campos grasslands of South America. In Uruguay, nearly 4 million ha (23% of the total land base) have been or will be impacted by afforestation. It is unknown how this recent afforestation will impact vegetation that has already adapted to long term grazing in the Campos. I examined how this management cycle affects vegetation species richness, frequency, and composition in eucalyptus plantations, studying five distinct phases of the management cycle (grassland, young forest, mid-stage forest, old forest, and post-harvest). At each of 25 sites, I recorded species presence in 40, 1 m^2 plots and categorized species by life form trait (annual graminoid, annual herb, perennial graminoid, perennial herb, woody, or fern). Impacts on species richness and frequency were greatest in mid-stage forests. Community composition also differed by phase (mid-stage forests were different from other phases) as well as sample date. While agroforestry changes the vegetation, there appears to be potential for post-harvest recovery. This research suggests that afforestation does affect vegetation richness and composition, but there is a potential for recovery. However, further research is needed to fully understand the effects of multiple rotations, effects on taxa other than plants, and consequences of landscape fragmentation across the Campos.

Introduction

Natural disturbances have long been seen as an important determinant of plant diversity (Hobbs & Huenneke 1992). Plant community diversity has been shown to respond to fire (Hobbs & Huenneke 1992), floods (Attiwill 1994b), and grazing (Milchunas *et al.* 1988). Less research has studied how multiple disturbances interactively affect species richness, especially anthropogenic disturbances (Valone 2003, although see Ross *et al.* 2004).

Uruguay, as well as southern Brazil and northern Argentina, comprise the Campos ecoregion, one of the most extensive grasslands globally. Most (85%) of Uruguay is considered natural grassland, dominated by perennial grass and herb species (Pallares *et al.* 2005). Grasslands are typically diverse and species rich (Alrababah *et al.* 2007). Annual species are often more common in grasslands than in other ecosystems, and there may be more invasive species relative to other land cover types (Papanastasis *et al.* 1995, Altesor *et al.* 1998, Berretta *et al.* 2000, Cingolani *et al.* 2003, Pallares *et al.* 2005, Altesor *et al.* 2006, Gibson 2009).

High growth rates and diversity of grassland vegetation make the Campos region an important area for livestock production, primarily cattle and sheep. Domestic herbivores were introduced during the sixteenth century (Paruelo *et al.* 2007). Currently, the Campos supports 10.1 million cattle and 13 million sheep. Therefore, grazing has had a long-term and continuing impact on this natural grassland ecosystem (Pallares *et al.* 2005) and has been a primary determinant of understory species composition and structure (Soriano *et al.* 1991, Altesor *et al.* 1998, Paruelo *et al.* 2007). Species respond individually to grazing disturbance, depending on each species' palatability to livestock and life history traits (Kohler *et al.* 2004, Scimone *et al.* 2007, Austrheim *et al.* 2008). A consistent, moderate level of grazing may actually enhance diversity (Olff & Ritchie 1998, Reitalu *et al.* 2010) as the predictability of the disturbance regulates plant community processes, and allows vegetation dynamics to be in relative equilibrium (Briske *et al.* 2003, Romermann *et al.* 2009). Heavy grazing may reduce diversity, if sufficiently severe to extirpate palatable plants from an area (Johnson 1956).

In the Campos ecoregion, and especially in Uruguay, afforestation is increasingly occurring on grasslands that have been subject to long-term grazing (Carrere & Lohmann 1996, Geary 2001, Baldi *et al.* 2006, Baldi & Paruelo 2008, Vega *et al.* 2009). Forest plantations are being established where none existed in recorded history. In Uruguay alone, 23% of the land base (approximately 4 million ha) has been designated for afforestation (Fig. 1.1); of this, nearly 1 million ha have already been planted (Uruguay XXI 2010). Landscape-scale forest habitat was not common prior to recent afforestation (e.g., Darwin 2009): native forests cover less than 4% of Uruguay's total land base (Geary 2001), mostly located along rivers and streams.

Afforestation can greatly impact understory vegetation. Light conditions, soil characteristics, and moisture regimes change throughout the rotation cycle (Farley *et al.* 2005, Buscardo *et al.* 2008). In general, native species richness decreases with afforestation (Bremer & Farley 2010). In the Northern hemisphere and Amazonian forests, species richness decreases in grassland to plantation transition and decreases further with plantation age (Buscardo *et al.* 2008, Bremer & Farley 2010). Brudvig & Damschen (2011) found that afforested sites that were previously pasture can have lower species richness than long-term forests, although the presence of fire when sites were pasture also undoubtedly influenced understory vegetation in this study. Some local extinctions and introductions may occur with afforestation, as species adapted to open environments may not persist as forest canopies close, and shade tolerant species may appear as the forest habitat develops (Loumeto & Huttel 1997).

Vegetation response to afforestation can vary by life form, lifespan, or origin. Herbaceous and grassland species cover may decrease with afforestation, as shown in the flooding pampas and mountainous areas of Argentina (del Pilar Clavijo *et al.* 2005, Cuevas & Zalba 2010). In contrast, tree and shrub species richness may increase with time following afforestation (Loumeto & Huttel 1997, Onaindia *et al.* 2004), although high fire frequency in the systems where these studies were conducted (savannas and woodlands of Congo and mixed woodlands of Spain, respectively) may also have contributed to this response. Some studies have found higher cover of introduced species with afforestation, and an overall decline in species diversity (del Pilar Clavijo *et al.* 2005, Dickie *et al.* 2011).

Little ecological research has been conducted in Uruguay until recently, and even now, our understanding of plant community dynamics is limited (e.g., see Gautreau 2010). The research conducted in Uruguay on this topic has focused on grazed grasslands (Altesor *et al.* 1998, Altesor *et al.* 1999, Texeira & Altesor 2009), but the combined effects of grazing and afforestation on understory vegetation have not been studied and there is little understanding of the effects of the short forest rotation cycles (15 years) being used in this region on vegetation dynamics. To understand the combined effects of long-term grazing and recent afforestation on understory vegetation over the agroforestry management cycle being employed in Uruguay, I used a space-for-time substitution (Pickett 1989), quantifying understory plant community composition across five afforestation management phases. Specifically, I have two hypotheses:

1. Afforestation, in addition to grazing, increases the disturbance level to the extent that total species richness declines, but species may have individual responses according to their life form trait, and

2. Species composition will change in response to changes in light and soil conditions and decreasing competition from surrounding vegetation as forests develop.

Methods

Study Area

Research was conducted in the departments of Tacuarembó and Rivera in northern Uruguay (Fig. 2.1). Average annual precipitation (mean annual precipitation 1979-2010) is approximately 1460 mm, with the greatest monthly average precipitation (165 mm) occurring in April, and the lowest in August (80 mm) (INIA 2011). Rainfall can be highly variable, and droughts can occur throughout the year (Berretta *et al.* 2000), though there is no prolonged dry season (Overbeck *et al.* 2007). Mean annual temperature ranges from 16 to 19°C, with mean summer temperatures ranging from 22 to 27°C, and mean winter temperatures ranging from 13.5 to 16°C (Berretta *et al.* 2000, Pallares *et al.* 2005). The soils of the area formed from loess deposits of unconsolidated silt and sand (Foth & Schafer 1980, Paruelo *et al.* 2007, Brady & Weil 2008). Soils are heterogeneous in the Campos, but in the study area, soils are generally fertile: mollisols are dominant, with alfisols in humid areas (Foth & Schafer 1980, Soriano *et al.* 1991).

Approximately 70% of the afforested land in Uruguay is planted with *Eucalyptus grandis* or *E. globulus*, and the majority of remaining land is planted with *Pinus* species (Uruguay XXI 2010). Many afforested areas have been planted with multiple species, separated into different plantations. Because *Eucalyptus* species tend to be frost intolerant, they are typically planted on the hill slopes, and *Pinus* species are planted in the valleys (J.P. Posse, Weyerhaeuser Uruguay, pers. comm.) Within the afforested landscape, 34% of the land is not planted; the majority of unplanted land is in low-lying, wet areas, firebreaks, fence rows, or at sites that currently support native forests.

Field Methods

Data collection occurred in May and November 2010, and May 2011. Study sites were in eucalyptus plantations, with similar elevation and located near one another. Study sites represented five phases of agroforestry management being applied to Weyerhaeuser Uruguay lands (Grassland, Young Forest, Mid-stage Forest, Old Forest, and Post-Harvest) (Table 2.1; Appendix A). Management phases differed in canopy cover, grazing, and recent silvicultural treatments. Grasslands (G) were unplanted locations within plantation areas that have been subject to long-term cattle grazing, and never previously managed for forestry. Young Forests (YF) were planted, on average, 10 months prior to study; at the time of study, trees were very small and basal area was minimal. At the YF sites, glyphosate had been applied directly to the furrowed rows where trees were planted but not between rows (rows were typically 5 m apart). Mid-stage forests (MF) were selected from stands planted 7 years prior to sampling. In addition to operational treatments described in YF, MF stands had been pruned and then opened to grazing 2 years after planting; 5 years prior to the sample date. Trees in MF stands were quite large, having achieved a diameter of nearly 25 cm in seven years. Stand basal area was much greater in MF than in YF. Old Forests (OF) were selected from sites thinned approximately 6 months prior to study, and were planted, on average, 13 years prior to study. These sites also were subjected to the operational treatments described for YF and MF. Most OF sites experienced some natural regeneration that resulted in a considerable number of small trees throughout the site. Because of this, average diameter and stand basal area in OF are similar to MF, although the trees planted originally are larger than those at the MF sites. Post-harvest sites (PH) were, on average, harvested 5 months prior to study. These stands were approximately 16 years old when harvested, and had been subject to all the stand treatments described for the earlier phases. Five replicates of each management phase were selected for this study (using ArcGIS to select sites based on appropriate stand age, planted species (all study sites were planted with *Eucalyptus grandis*), soil type (5A, characterized by deep sandy soils of low fertility; (Berretta 2003)), and location within the western Tacuarembó or Rivera regions. One replicate was sampled in May 2010, and two replicates were sampled in both November 2010 and May 2011.

Within each study site, a modified transect-grid was established in a 1.44-ha area adjacent to the stand boundary (typically a road or adjacent stand, for ease of access); sites were 1.44 ha in size to allow for a 1-ha sampling area with a 10 m buffer on all sides. Four transects (100 m in length) were established within each site; each transect was placed 30 m from and parallel to the closest sample area edge. Transects were oriented according to the closest stand boundary, and therefore not always parallel and perpendicular to tree rows. Along these transects, 1 m^2 quadrats were spaced at 10 m intervals (n = 40 per site) (Fig. 2.2). In YF, because of bare soil and direct herbicide application to planting furrows, if a quadrat landed in a furrowed row, it was moved 1 m outside the furrow. I also assessed the effects of herbicides on vegetation in furrowed rows by comparing species richness in quadrats that fell within a furrowed row with plots adjacent to these, 1 m outside the furrow, on a subset of quadrats within two YF sites, and found no difference in species richness. Nonetheless, only the plots moved outside the furrows in YF stands were used in the analyses described below.

Within each quadrat, I recorded the presence of every species. Species identifications proved challenging due to the dearth of botanical research conducted in this region, and as a result, several identifications could only be made to broader categories (family or growth form). Species that could not be identified were given unique codes; unknowns were included in total species richness calculations, but were excluded from further analysis if they could not be assigned to a life form category. Life form categories were based upon Zuloaga et al. (2008); species were categorized into one of six potential life forms: annual graminoid, perennial graminoid, annual herb, perennial herb, fern, or woody species. Species were also summarized by origin: native or introduced.

Analysis

I tested for differences among management phases in total species richness, the proportional importance of life form categories, and composition. Total species richness was calculated as the total number of unique plants encountered across all quadrats within each site. I used generalized linear model (GLM, proc genmod) to test the effects of agroforestry management phase (G, YF, MF, OF, or PH) and sample date (May 2010, November 2010, or May 2011), and the phase x sample date interaction on total richness. Richness was log-transformed as for a Poisson distribution since it was count data. The analysis was conducted in SAS 9.2, with phase, sample date, and the phase × sample date interaction as fixed effects (Zar 1999, Littell *et al.* 2006). Post-hoc comparisons for significant differences were determined using least squared means. I set $\alpha = 0.05$ for all analyses.

Proportional importance was assessed in three ways: relative richness, relative frequency, and mean species frequency. These indices were calculated separately for each life form category, and provide different insights into the properties of the vegetation. Relative richness was calculated as the number of species of a given life form category as a proportion of the total species richness; larger values indicate that proportionally more of the species belong to that life form category. Relative frequency was calculated as the proportion of plots within each site that contained a species of a given life form category; larger values indicate that that life form category was more widespread within the site. Since relative frequency is sensitive to the number of species within a life form category, I also calculated mean species frequency as the mean number of occurrences of each species of a given category at a site. Larger values of this variable indicate that the average species in this life form category was more widespread within the site. Proportional data were transformed using a link-log transformation for binomial distributions and were analyzed using GLMs as described above. Species richness for ferns was very low in general, and models for this life form category would not converge unless the interaction term was removed. Post-hoc comparisons for significant differences were determined using least squared means.

The effects of agroforestry management on understory community composition were analyzed using Permutational multivariate analysis of variance (PERMANOVA). I conducted PERMANOVA through the Adonis function in R which uses sequential sums of squares. Rare species (present in < 5% of plots) were excluded from this analysis; 159 species were included. Data were relativised by species maxima and site totals (McCune & Grace 2002). The Bray-Curtis distance measure was used, and 9999 permutations were run. I tested the effects of phase, sample date and phase x sample date species composition, and conducted post-hoc comparisons using orthogonal contrasts to determine significant differences between phases and sample dates: Grasslands to Young Forests (G:YF), Grasslands to Mid-stage Forests (G:MF), Grasslands to Old Forests (G:OF), Grasslands to Post-Harvest (G:PH), May 2010 to November 2010 (May10:Nov10), and May 2010 to May 2011 (May10:May11). The compositional analysis was visualized using nonmetric multidimensional scaling (NMDS) ordination, using the Bray-Curtis distance measure, 3 axes, and calculating stress using monotone regression, running a maximum of 40 times. Convergence was met after 26 runs, with a stress of 0.150068. The non-metric fit of the ordination was $R^2 = 0.9777$; the linear fit was $R^2 = 0.843$. All compositional analyses and ordinations were conducted in R v2.14.2.

Results

In total, I recorded 218 unique plants in our study; 79% were identified to species, 83% to genus and 88% to family (Appendix B). Life form category could be assigned to 80% of our plants; of those, 49% were perennial herbs, 21% were perennial graminoids, 14% were annual herbs, 11% were woody, 3% were annual graminoids, and 2% were ferns. Origin was identified for 74% of species; of these, 94% were native and 6% were introduced. Introduced species were found in every phase, in varying relative abundances: introduced species comprised 2.7% of all species identified by origin in G, 4.3% in YF, 2.1% in MF, 6.4% in OF, and 6.5% in PH. In absolute terms, there were an average of 1.4 introduced species per site in G, 2.4 in YF, 0.4 in MF, 2 in OF, and 2.2 in PH.

Site-level total species richness differed significantly among phases and phase x sample date (Table 2.2). G and YF phases had significantly more species than other phases, and species richness was lowest in MF (Fig. 2.3; also see Appendix C). The date of sampling did not significantly affect total species richness, although the phase x sample date interaction was significant because of temporal variability in richness in OF and PH; for May2010 sample date, richness in G and YF was similar to OF and PH but this was not the case in later samples.

Species composition differed by phase and sample date (Table 2.3, Fig. 2.4). Contrasts revealed that composition did not differ between G and YF, but differed between G and all other phases. YF differed from MF and OF in composition but not from PH. Additionally, MF and PH were significantly different. Composition differed between all sample dates.

Relative species richness differed by agroforestry management phase, sample date, and phase x sample date for most trait categories (Table 2.2). Perennial graminoids and herbs were the most species rich categories in all phases, although their relative richness differed among phases (Fig. 2.5). Perennial herbs accounted for a much higher proportion of the species in MF than in other phases. Annual graminoids had greatest relative species richness in YF and PH, although PH was also similar to G and OF. Annual herbs had similar relative species richness in all phases but MF, where relative species richness was low. Ferns had the greatest relative species richness in OF and least in YF. Woody species had a greater relative richness in G than any other phase.

Relative frequency was significantly different by phase for all response variables, by sample date for most response variables (except ferns and perennial graminoids), and by phase x sample date interaction for those that could be tested (Table 2.2). Perennial herbs and graminoids had the highest mean plot frequency, but both declined in frequency from G to other phases (Fig. 2.6). While perennial herbs declined from G to YF and again to MF, OF, and PH, perennial graminoids showed some sign of recovery and plot frequency was greater in OF and PH than MF. Annual graminoids and herbs had the highest plot frequency in G and YF, and least in MF. Ferns had the highest plot frequency in OF. Woody species were most frequent in G and least in MF, OF and PH.

Mean species frequency differed significantly by phase for most life form categories, but sample date and phase x sample date effects were less important than for other variables (Table 2.2). In general, species were fairly uncommon: they were present in less than a third of the plots within a study site (Fig. 2.7). Annual graminoids were most common in YF and G, and least common in MF. Perennial graminoids and herbs were most common in G and YF, and declined in MF, OF, and PH. Annual herbs were least common in MF, but were similar in all other phases. Woody species were most common in G, and ferns were most common in OF.

Discussion

Total richness and composition

In general, there was a decrease in total species richness following afforestation; this coincides with previous research that demonstrates that vegetation richness decreases with increasing tree cover

(Alrababah *et al.* 2007). Total richness was highest in G and YF, when there is higher light availability; richness was lowest in MF, likely due to low light conditions (Buscardo *et al.* 2008). In addition, litter accumulation on the forest floor could impede seeds from reaching the soil layer, and may provide a physical barrier to seedling and sprout emergence (Facelli & Pickett 1991, Wilby & Brown 2001). Forests also have greater evapotranspiration, potentially reducing soil moisture (Farley *et al.* 2005); this decrease in moisture availability may also negatively affect species richness, especially in dense MF (Nosetto *et al.* 2005). I saw an increase in species richness between MF and older phases (OF and PH), which coincides with previous research (Cuevas & Zalba 2010) and may be due to an increase in light availability after thinning (OF) and harvest (PH) (Buscardo *et al.* 2008).

Plant community composition is heavily influenced by previous land-use history (Ito *et al.* 2004, Baum *et al.* 2009). At my study sites, only species adapted to livestock grazing likely were present at the time of afforestation. As a result, species poorly adapted to grazing would be relatively rare in this system and would have little opportunity to colonize ungrazed forest habitats in the relatively short time period when livestock are excluded (YF phase). The primary driver of the vegetation differences among management phases observed in our study, therefore, is afforestation (Alrababah *et al.* 2007).

I also found a difference in composition by sample date, which may be partially attributed to sampling expertise and spatial correlation between sites. I attempted to minimize sampling bias between sample dates by using a small field crew, and working closely together at all sites. However, I gained taxonomic expertise each sample date, and my ability to identify vegetation to species did increase, and although I was able to later identify many unknown plants encountered on early sample dates, there were still more unknowns in earlier samples than later. Also, I attempted to locate sample sites in each sample date close together to make sampling efficient. Therefore, some of the differences between sample dates may be partially due to spatial separation among the sites sampled in different seasons. Because neither of these circumstances resulted in a significant sample date x phase interaction (the difference in richness or composition by phase varied by each sample date), I do not think changes in taxonomic expertise or potential spatial correlation among some sites has important implications for my study.

Life form traits

My results differ from other studies in that very few exotic species occurred in this system, especially in afforested phases, and of these exotics, some were actively introduced to provide livestock forage (M. Bemhaja, INIA, pers. comm.). Of the species I encountered in this study, 94% are native to southern

South America and 26% are endemic to the region (southern Brazil, Chile, Argentina, Paraguay, and Uruguay). As with the native species at my study sites, these exotic species are adapted to the grazed grassland environment by exhibiting resistance (i.e., avoidance) or tolerance (i.e., capacity for re-growth) to livestock (Evju *et al.* 2009). The fact that only exotic species with the capacity to adapt to high intensity grazing can persist in this environment may be one reason why the representation of non-native species at our study sites was low.

I also saw several changes in vegetation across the agroforestry management cycle by life form trait: annual species seem more sensitive to afforestation than perennials (similar to findings by Baum et al. (2009), potentially because of litter accumulation on the forest floor impeding seedling emergence of annuals (Facelli & Pickett 1991, Wilby & Brown 2001). I also generally found a decrease in woody species with afforestation, which was surprising. Much research has found an increase in woody species with afforestation, as the change in light availability and soil characteristics reduces competition from other growth forms (Powers *et al.* 1997). However, in most previous research of afforestation, it is the only disturbance in the system studied. In the Campos, afforestation has been introduced as a new disturbance on top of an already disturbed (grazed) ecosystem. The combined effect of grazing and afforestation on vegetation dynamics in mixed forest-grassland ecosystems, like the Campos are likely more complex than in systems influenced by only afforestation, so comparisons with other studies on this topic may not be meaningful (House *et al.* 2003).

Potential recovery

Species richness and composition declined between G and MF phases but exhibited signs of potential recovery following forest thinning with richness increasing from MF to OF and PH sites. Composition in PH was similar to YF (which was also similar to G). Vegetation in the Campos ecoregion may be more resilient to impacts of afforestation than in other regions, given that plants have already adapted to long-term grazing (Greenberg *et al.* 1995, Li *et al.* 2007). Many adaptations developed in a grazed system, such as wind-dispersed seeds and versatile rosette morphology, also serve plants well in afforested systems, where wind dispersed seeds can colonize open spaces, and the versatile architecture of rosettes (flat structure when not flowering) can take better advantage of resources, like reduced light (McIntyre *et al.* 1995). With these adaptations, plants can respond to the individual and interactive effects of multiple disturbances (Savadogo *et al.* 2009)

Plant species adapted to forest conditions are likely rare in the Campos ecosystem. Native forest cover occurs primarily along water courses. Therefore, there was limited availability of forest understory plants to colonize afforested sites. In addition, short rotation cycles may hamper the establishment of forest species especially considering the rapid changes in canopy conditions associated with thinning and harvest (Brockerhoff *et al.* 2008). The short rotations, pruning and thinning of the plantation stands also may favor grassland species. Grassland species are present during some of the management phases, especially early in the rotation (YF) and after thinning (OF). Therefore, some grassland plants may persist through the rotation cycle. Even grassland species that cannot persist under a forest canopy may rapidly recolonize a site following harvest from stored seed or through seed dispersal and colonization of a site after tree harvest (Eycott *et al.* 2006).

The continued presence of grassland patches within the plantation forest landscape may facilitate recovery of plant diversity following an afforestation cycle (Baum *et al.* 2009). In my study area, generally half the land within afforested areas is planted; the remaining land remains in grazed grassland (J. Posse, Weyerhaeuser Uruguay, pers. comm.). The swaths of grassland maintained within the afforested landscape provide a source of propagules to recolonize afforested sites once conditions become suitable for these species. However, these remnant patches often possess physical characteristics different from forested sites; often, grasslands occupy valleys and may have wetter soils than forests. Thus, the suite of remaining species present to colonize adjacent forests may be somewhat different than the historical grassland vegetation. The spatial design of the plantations and remnant grasslands may be a major factor in determining future composition of understory vegetation through multiple rotations. Eventually, however, multiple short rotations may result in decreased species richness and diversity (Wen *et al.* 2010).

My results reflect changes in plant communities associated with only the first rotation of trees. Vegetation may continue to evolve over multiple rotations as species incapable of adapting to forested conditions are reduced in abundance. The full impacts of afforestation will not be fully understood until both the first rotation effects, examined in this study, as well as the changes associated with repeated cycles of tree planting and harvesting are examined. Because of the positive socioeconomic impacts of afforestation in Uruguay, including increased employment opportunities and wages (WFO 2002, Olmos & Siry 2009), it is likely that afforestation will have a long-term presence in the area, and it is critical that more research is conducted to understand the combined impacts of long-term grazing and afforestation in the Campos.

Acknowledgements

I thank Cat Adams and Scott Batiuk for assistance with field sampling, Juliana Ingaramo, Felipe Irureta, and Juan Pedro Posse for assistance with site selection, and Steve Duke for statistical guidance. María Bemhaja, Martin Juarena, and especially Eduardo Marchesi provided invaluable contributions to species identifications and my understanding of grasslands in Uruguay. Funding was provided by Weyerhaeuser Global Timberlands Technology.

Table 2.1. Site characteristics for each agroforestry management phase. Operations include any operational activity that sites have indergone in the current and previous agroforestry management phase. Mean stand age is the mean age of stands within each phase, at the time of study. Tree diameter and stand density are mean values of sites within each phase.

	Grassland (G)	Young Forest (YF)	Mid-stage Forest (MF)	Old Forest (OF)	Post-Harvest (PH)
Trees	Absent	Present	Present	Present	Absent
Grazing	Present	Absent	Present	Present	Present
Operations		Planted	Planted Pruned	Planted Pruned Thinned	Planted Pruned Thinned Harvested
Stand age		10 mo	7 yr	13 yr	16 yr pre-harvest, 5 mo post-harvest
Tree diameter (cm)	0	4.0	26.8	27.6	0
Stand density (tph)	0	337	351	312	0
Basal area (m^2/ha)		0.9	43.0	42.2	0

Table 2.2. Effects of phase, sample date, and phase x sample date for total and relative richness, relative frequency, and mean species frequency for life form category. Analyses were conducted using a generalized linear model that uses maximum likelihood estimators. Significant results are bolded.

		Phase			Sample date			Phase x Sample date		
		DF	Chi-square	*P*-value	DF	Chi-square	*P*-value	DF	Chi-square	*P*-value
Total richness	Richness	4	92.24	**<0.0001**	2	2.69	0.2603	8	26.21	**0.0010**
Relative richness	Annual gram	4	63.26	**<0.0001**	2	10.37	**0.0056**	8	22.36	**0.0043**
	Annual herb	4	32.07	**<0.0001**	2	72.51	**<0.0001**	8	52.30	**<0.0001**
	Fern	4	112.31	**<0.0001**	2	10.58	**0.0051**			
	Perennial gram	4	1.99	0.7368	2	3.07	0.2159	8	13.29	0.1021
	Perennial herb	4	51.79	**<0.0001**	2	3.29	0.1934	8	7.64	0.4699
	Woody	4	49.87	**<0.0001**	2	11.65	**0.0029**	8	37.80	**<0.0001**
Relative frequency	Annual gram	4	125.72	**<0.0001**	2	13.43	**0.0012**	8	45.11	**<0.0001**
	Annual herb	4	115.34	**<0.0001**	2	113.03	**<0.0001**	8	118.16	**<0.0001**
	Fern	4	104.47	**<0.0001**	2	2.93	0.2309			
	Perennial gram	4	229.78	**<0.0001**	2	0.00	1.0000	8	47.89	**<0.0001**
	Perennial herb	4	124.56	**<0.0001**	2	25.29	**<0.0001**	8	7.64	0.4699
	Woody	4	192.17	**<0.0001**	2	17.28	**0.0002**	8	50.75	**<0.0001**
Mean species frequency	Annual gram	4	81.87	**<0.0001**	2	5.66	0.0589	8	48.4	**<0.0001**
	Annual herb	4	15.36	**0.0040**	2	25.55	**<0.0001**	8	25.78	**0.0011**
	Fern	4	70.61	**<0.0001**	2	1.22	0.5440			
	Perennial gram	4	23.63	**<0.0001**	2	0.79	0.6739	8	12.67	0.1236
	Perennial herb	4	15.29	**0.0041**	2	0.60	0.7422	8	5.94	0.6544
	Woody	4	39.33	**<0.0001**	2	4.39	0.1113	8	10.54	0.2291

Table 2.3. Effects of agroforestry management phase, sample date, and phase x sample date on vegetation composition. Results were determined from PERMANOVA; significant results are bolded.

	DF	Sums of Squares	Mean Squares	F.Model	R^2	*P*-value
Phase	4	1.7791	0.44476	1.48972	0.20274	**0.0016**
Sample date	2	1.7312	0.86558	2.89924	0.19728	**0.0001**
Phase x Sample date	8	2.2794	0.28492	0.95434	0.25975	0.6670
Residuals	10	2.9855	0.29855		0.34023	
Total	24	8.7751			1.00000	

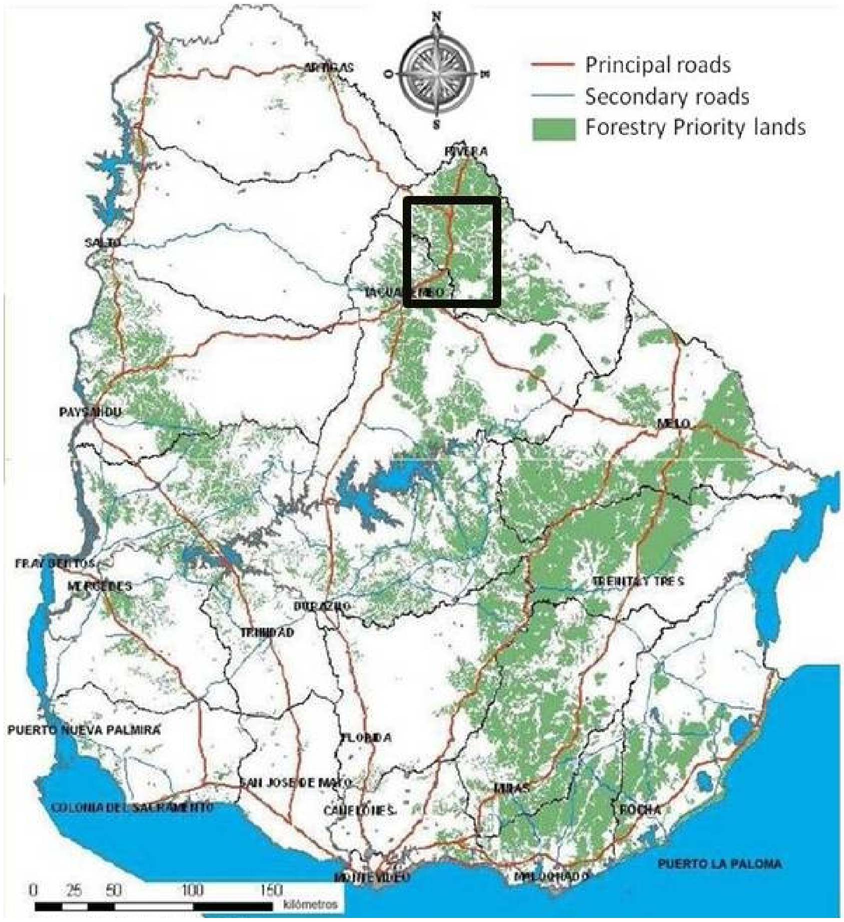

Figure 2.1. Map of Uruguay, including areas designated as Forestry Priority (areas that have been or will be afforested). As of 2010, 24% of this area has been planted; almost half (46%) of the planting has occurred in the North Central area of Uruguay, where our research occurred (25 study sites within the area indicated by the black square) (Uruguay XXI 2010).

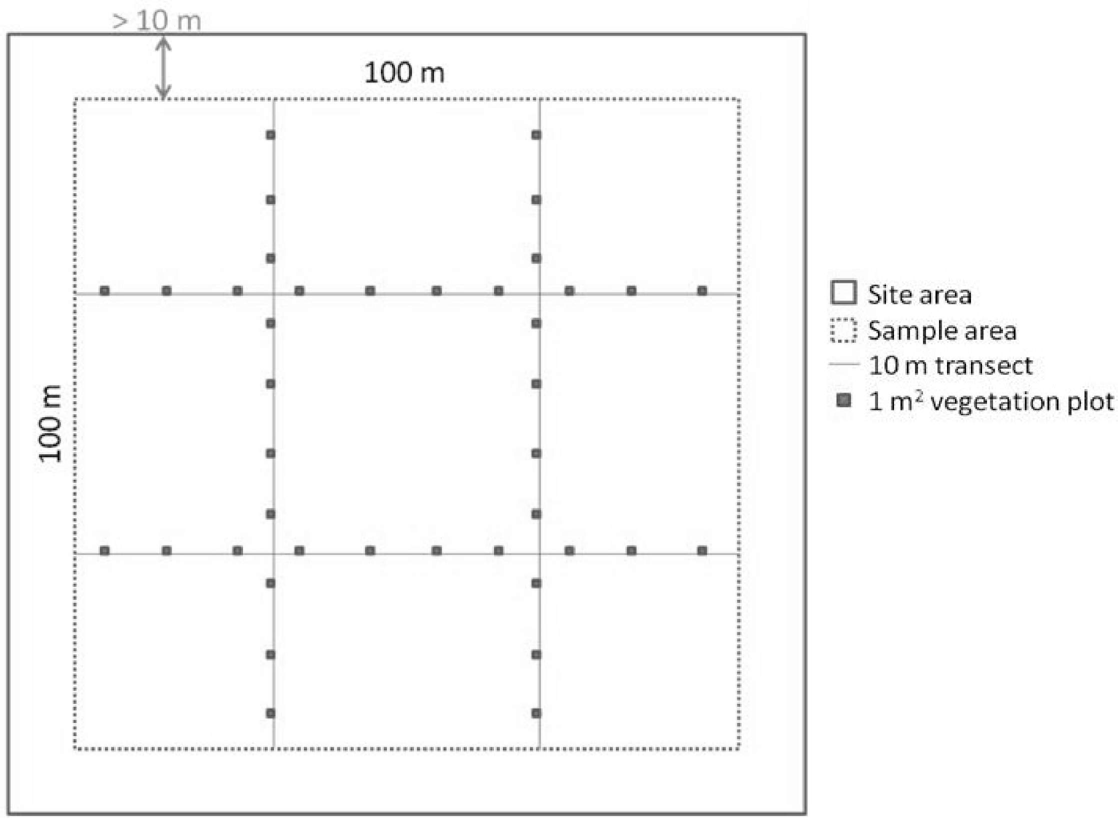

Figure 2.2. Sampling design employed at each study site to determine vegetation species richness at by agroforestry management phase: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH). Species presence was recorded in 40, 1 m^2 plots at each site, systematically arrayed on 4 transects.

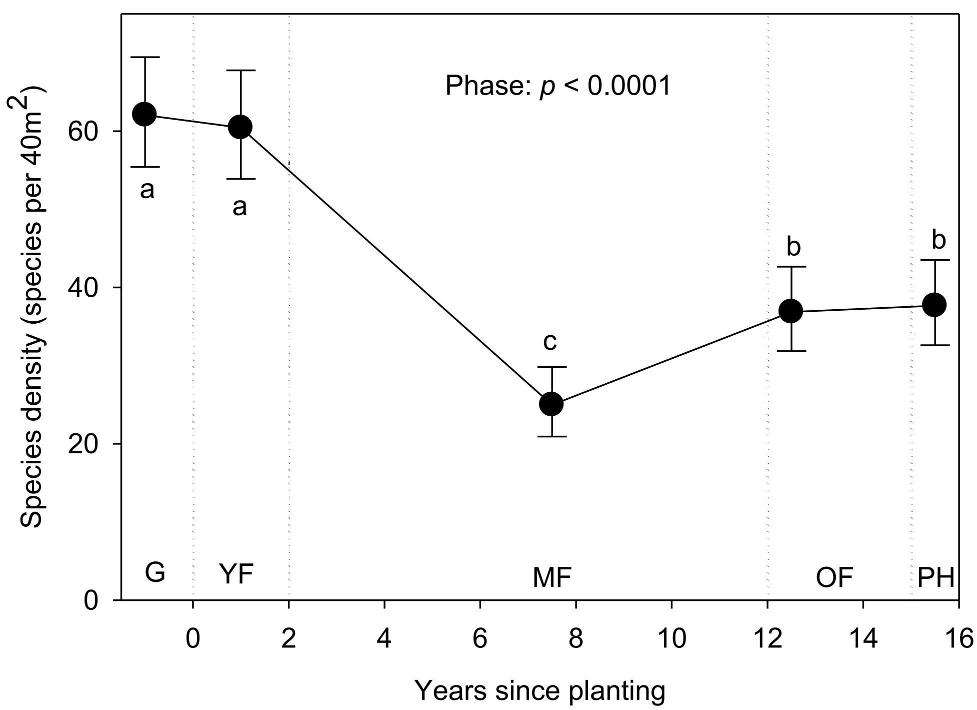

Figure 2.3. Total site-level species richness, represented by means with 95% confidence intervals, as a function of agroforestry management phase: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH). Agroforestry management phases are delineated by dotted lines. Differences in least squared means are denoted by lowercase letters.

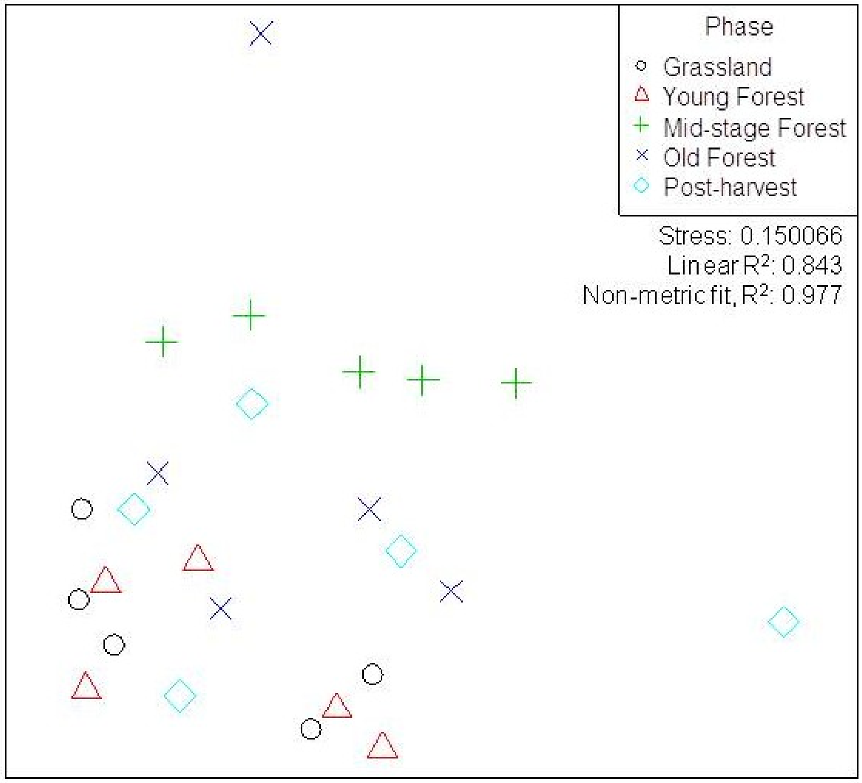

Figure 2.4. Vegetation community composition by agroforestry management phase. Data points are sites in species space. NMDS 2-dimensional display of a 3-dimensional ordination. Composition in G was different than MF (p = 0.0002), OF (p = 0.0105), and PH (p = 0.0047). Composition in YF also differed from MF (p = 0.0005) and OF (p = 0.0502). Also, composition differed between MF and PH (p = 0.0309).

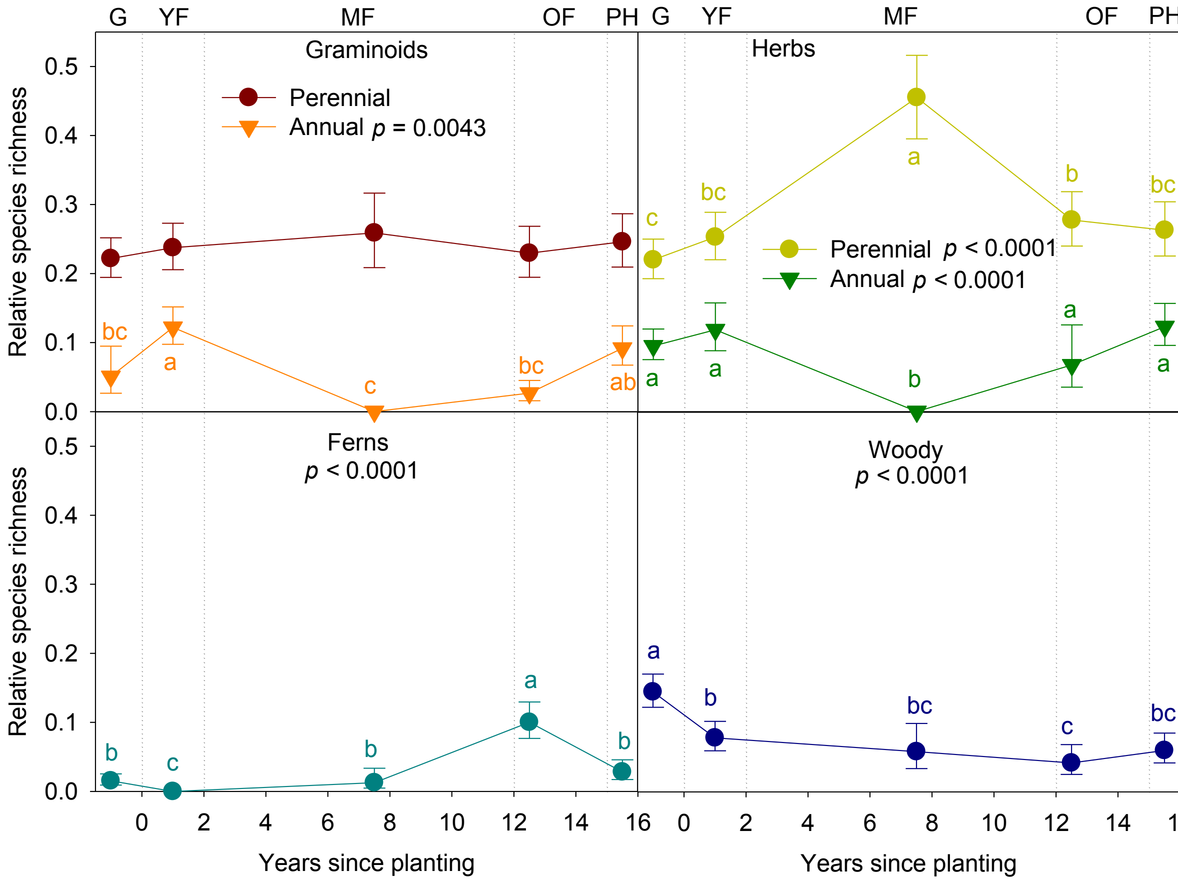

Figure 2.5. Relative species richness (proportion of total richness for each life form category) as a function of agroforestry management phase: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH). Agroforestry management phases are delineated by dotted lines and labeled above the graphs. Data points represent site-level means with 95% confidence intervals.

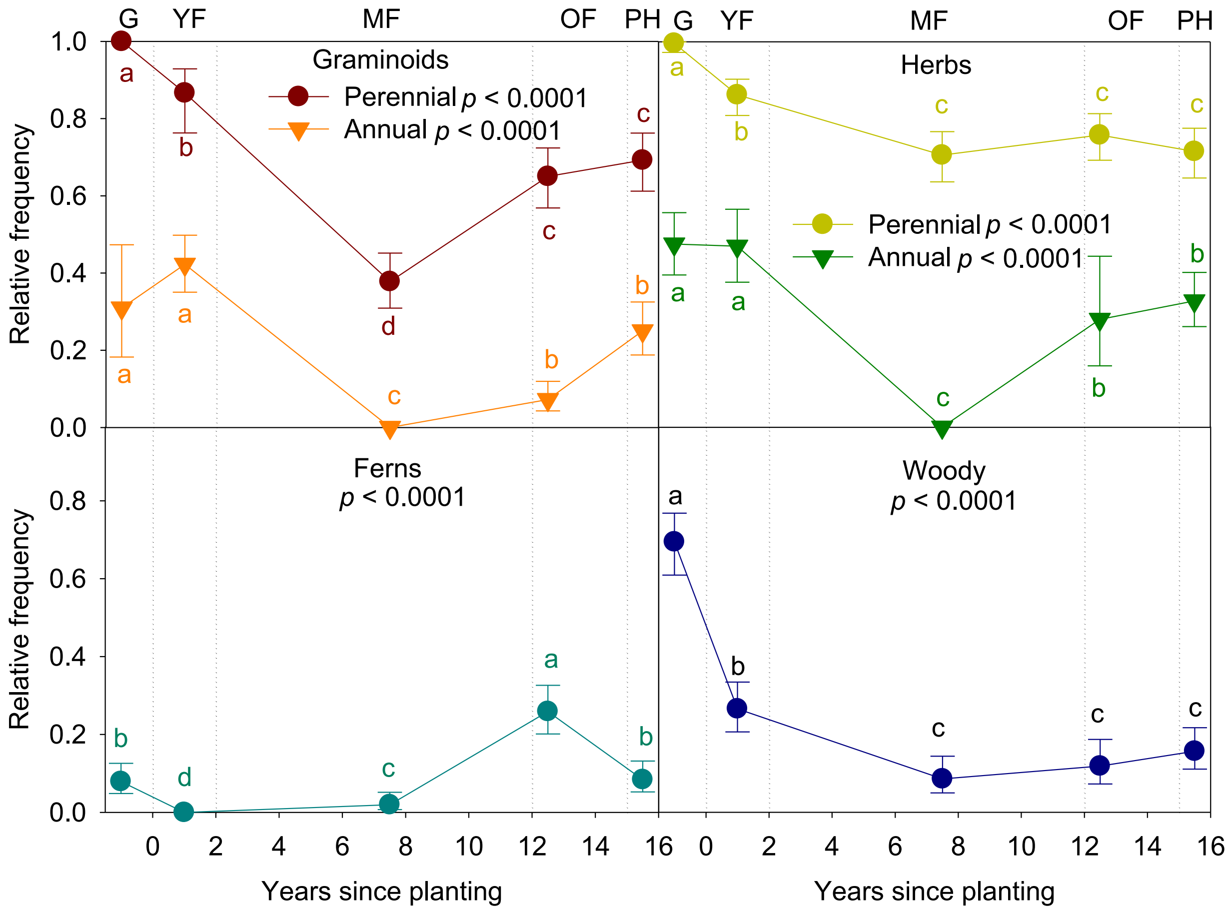

Figure 2.6. Relative frequency of life form categories (proportion of quadrats containing at least one species of that category) as a function of agroforestry management phase: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH). Agroforestry management phases are delineated by dotted lines and labeled above the graphs. Data points represent site-level means and 95% confidence intervals.

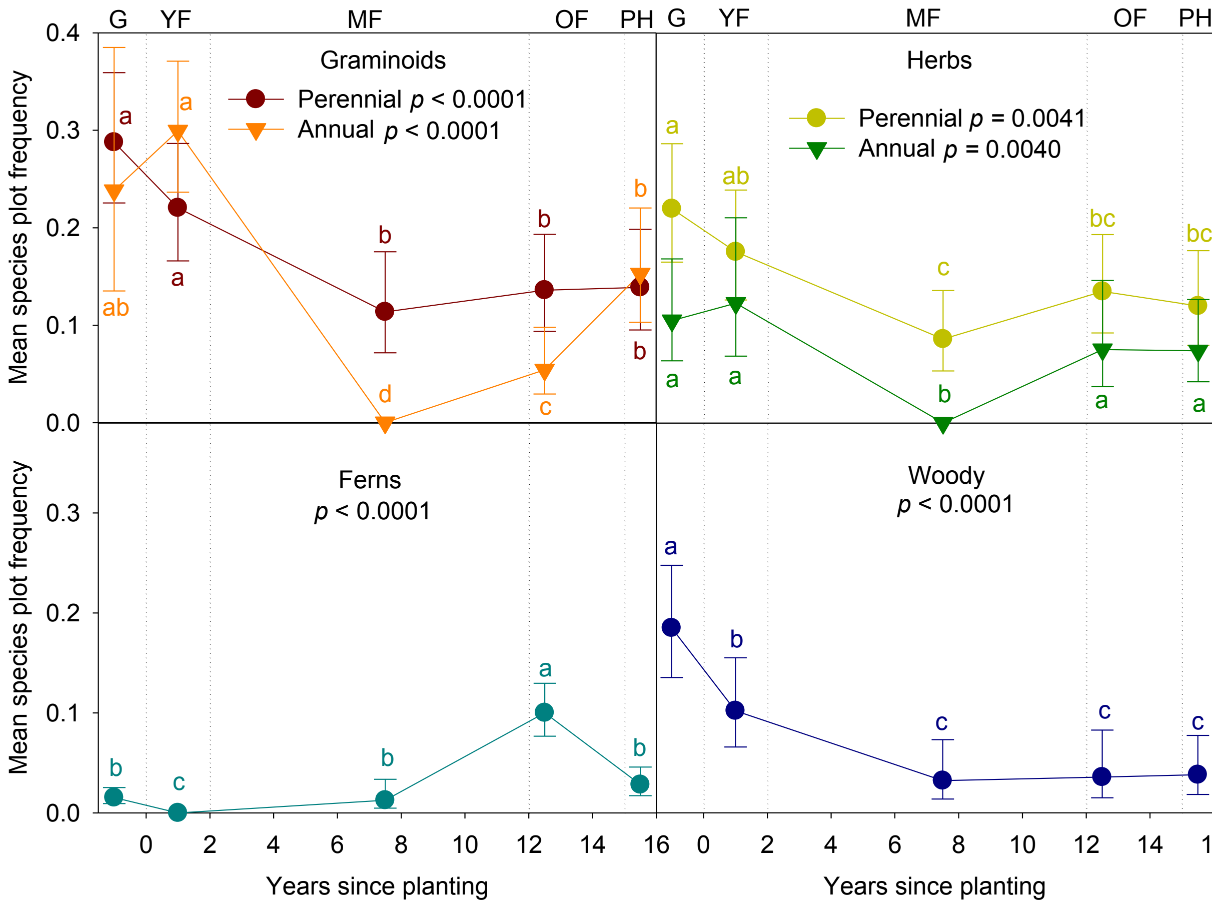

Figure 2.7. Mean species frequency of life form categories (total number of occurrences of species of a given category divided by the total number of species from that category recorded at a site) as a function of agroforestry management phase: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH). Agroforestry management phases are delineated by vertical dash lines and labeled above the graphs. Data points are site-level means with 95% confidence intervals.

Chapter 3
Soil characteristics through first-rotation Eucalyptus plantation cycle in Uruguay

Abstract

Land use has long-term effects on soil properties, and as land use changes, legacies from previous habitats or management may still impact soils. The majority of Uruguay, as part of the Campos ecoregion, is long-term grazed grassland, but afforestation is becoming a widespread disturbance. The effect of this major land use change on soil properties is not clear. I measured physical (moisture and bulk density) and chemical (pH, organic matter, and nutrient bases (total N, Al, Mg, Ca, K, and Na)) soil properties in five phases of agroforestry management of *Eucalyptus grandis* plantations: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH), and tested the effects of depth (0-5, 5-15, or 15-60 cm), phase, and depth x phase on each soil property. Neither soil moisture nor bulk density varied by any effect. While pH was similar across phases at the shallow depth, pH was lower in OF and OF and PH in mid- and deep soil depths. Organic matter and total N content decreased with soil depth. Most chemical variables (Al, Mg, Ca, and Na) differed by depth x phase, but significant differences generally revealed only differences between depths within the same phase. Long-term grazing at these sites likely reduced soil fertility prior to afforestation. Also, more changes may be expected in the future as these short-rotation plantations are subsequently replanted and harvested. Most soil variables were highly variable, and the sample size chosen for this study may have been too small to adequately demonstrate changes in soils characteristics with agroforestry management phase. This study highlights the highly variable nature of soils characteristics, and the need for future research to fully understand short-term impacts of afforestation and the response of soils to this major land use change.

Introduction

Soil provides stability and space for a plant's rooting structure, and provides water and nutrients for a plant to function (Kimmins 1997). Soil is affected by climate, vegetation, animals, topography, land management, and parent material, all of which can vary or weather with time (Gibson 2009). Land use can have long-term effects on soil properties, and as land use changes, legacies from previous management regimes may still impact soils (Dupouey *et al.* 2002). Soil nutrition and physical properties determine, at least partially, the type of vegetation occupying a site and the trajectory a plant community may follow during recovery from a disturbance. Therefore, plant community processes may be altered by changes in soil properties caused by management (Paul *et al.* 2002, Browning *et al.* 2008).

The Campos is one of the most extensive grasslands globally, covering large areas of Uruguay, southern Brazil and northern Argentina. This region has a long history of livestock grazing. Domestic herbivores were introduced during the sixteenth century (Paruelo *et al.* 2007), and now, the Campos supports 10.1 million cattle and 13 million sheep, resulting in a long-term impact on natural grasslands (Pallares *et al.* 2005). In the Campos ecoregion, and especially in Uruguay, afforestation is occurring rapidly on these grazed grasslands (Carrere & Lohmann 1996, Geary 2001, Baldi *et al.* 2006, Baldi & Paruelo 2008, Vega *et al.* 2009). Forest plantations are being established where none existed in recorded history. In Uruguay alone, 23% of the land base (approximately 4 million ha) has been designated for afforestation; of this, nearly 1 million ha have already been planted (Uruguay XXI 2010). Landscape-scale forest habitat was not common prior to recent afforestation (e.g., Darwin 2009): native forests cover less than 4% of Uruguay's total land base (Geary 2001), mostly located along rivers and streams.

Converting grasslands to forests may impact soil properties through changes in litter input, nutrient uptake and root distribution. The effects of this new management regime on soil properties of the Campos have not been fully assessed, however, and substantial alterations are possible. As grasslands are converted to forests, evapotranspiration and rainfall interception usually increase (Farley *et al.* 2005, Nosetto *et al.* 2005). Stream flow and runoff typically decrease as a result of afforestation, but the supply of water from forests usually is more temporally stable than in grasslands (Farley *et al.* 2005, Nosetto *et al.* 2005). Other research has shown that total soil moisture may not vary significantly among different land use types, but that variations in soil moisture with depth may be significant (Venkatesh *et al.* 2011).

Soil bulk density may be unaffected for a period of time after afforestation of grassland, at least in surface soils (Davis & Condron 2002, Evrendilek *et al.* 2004). However, if enough soil organic matter from the litter layer is incorporated into the soil during forest development, bulk density may decrease (Shirato *et al.* 2004, del Pilar Clavijo *et al.* 2005, Korkanc *et al.* 2009). Because plantations in the Campos are fast growing and have short rotation cycles, it remains unclear if organic matter from forest litter will impact bulk density (Parrotta 1999).

Long-term grazing in the Campos has likely reduced soil fertility (Garcia-Oliva *et al.* 1994) although soil conditions are likely very heterogeneous due to widespread waste deposition (Fernandez-Gimenez &

len-Diaz 2001). Converting grasslands to forests may reduce soil organic matter due to changes in root distribution (Booth *et al.* 2005), although this is not always found. Some research has shown that most roots of grasslands and forest plantations occupy the same soil depths (less than 0.5 m) (Schulze *et al.* 1996, Schenk & Jackson 2002), although this is primarily due to the fine root distribution of trees in shallow soils (Chen *et al.* 2000). Others research, has concluded that root distribution may be shallower in grasslands compared to forests (Jackson *et al.* 1996), primarily due to coarse roots of trees, and this can lead to differences between grassland and forests in soil characteristics by depth.

While changes in soil properties with forest development may be relatively well understood, the afforested sites in the Campos ecoregion are further complicated by its long-term grazing history. At present, no extensive research has been conducted to characterize changes in soil moisture, bulk density, and nutrition over the course of an agroforestry management cycle. To better understand the affects of afforestation and continued grazing on soil properties, I had two main objectives: 1) compare physical properties (soil moisture and bulk density), and 2) chemical properties (pH, organic matter and elemental concentrations of nitrogen, aluminum, calcium, magnesium, potassium, and sodium) at various soil depths among five agroforestry management phases (Grassland, Young Forest, Mid-stage Forest, Old Forest, and Post-Harvest).

Methods

Study area

Research was conducted in the Tacuarembó and Rivera regions of northern Uruguay (for details, see Chapter 2: Understory vegetation changes over an agroforestry management cycle in Uruguay). Approximately 70% of the afforested land in Uruguay is planted with *Eucalyptus grandis* or *E. globulus*, and the majority of remaining land is planted with *Pinus* species (Uruguay XXI 2010). Industrial plantations are generally mono-specific but species do vary among plantations. Because *Eucalyptus* species tend to be frost intolerant, they are typically planted on the hill slopes, and *Pinus* species are planted in the valleys (J.P. Posse, Weyerhaeuser Uruguay, pers. comm.) Within the afforested landscape, 34% of the land is not planted; the majority of unplanted land is in low-lying, wet areas, firebreaks, fence rows, or at sites that currently support native forests.

Field and laboratory methods

Data collection occurred in the autumn of 2011. Study sites were in *Eucalyptus grandis* plantations, with similar elevation and located in close proximity to one another. Study sites represented five phases of agroforestry management being applied to Weyerhaeuser Uruguay lands (Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH)) (for further details, see Chapter 2: Understory vegetation changes over an agroforestry management cycle in Uruguay). I chose study sites using ArcGIS, selecting sites based on appropriate stand age, planted species (all study sites were planted with *Eucalyptus grandis*), soil type (5A, characterized by deep sandy soils of low fertility; (Berretta 2003)), and location within the western Tacuarembó or Rivera regions.

Soil moisture and bulk density samples were collected from 3 sample depths (shallow: 0-5 cm, mid: 5-15 cm, and deep: 15-60 cm) at 3 sample points along 3 transects (following the same site layout at in Chapter 1), at 19 study sites (3 complete replicates; the 4th replicate was missing MF) (n = 171). Depths were chosen to be consistent with research previously conducted in Uruguay by INIA (Instituto Nacional de Investigación Agropecuaria) (E.P. Gomar, INIA, pers. comm.). After soils were dried, soil samples were pooled into two samples (based on proximity of sample locations). Soil pH, organic matter (%C), total nitrogen (%N), Aluminum (Al), Calcium (Ca), Magnesium (Mg), Potassium (K), and Sodium (Na) were determined from these pooled samples (n = 114).

Samples were collected within a single week and stored in moisture-proof bags after being weighed in the field. Samples were stored at the INIA lab in Tacuarembó, UY until analyses were conducted. Samples were dried at 105°C for 24 hrs, and then weighed again. Soil moisture was calculated as the difference between wet and dry weights (expressed as % moisture) for each sample. Because of an error in moisture calculation, two samples were excluded from analysis for soil moisture (n = 169). Bulk density was calculated by multiplying the volume of the cylinder used for sampling (5 cm diameter, 5 cm in height) by the dry weight of each sample (expressed in g/cm^3).

Soil pH was determined using 20 g of soil mixed with 50 mL of H_20. This solution was centrifuged for 3 minutes and allowed to stand for 15 minutes, and then pH was measured with an electrode on the supernatant of the extract. Soil organic matter and total N were calculated with a dry combustion (at 900°C) and subsequent detection of infrared CO_2 (for organic matter) and by thermal conductivity of N_2 (for total N) using a TruSpec LECO (CN628) elemental analyzer. For bases (Al, Ca, Mg, K, and Na), 5 g of soil was agitated in 100 mL of H_20, and then mixed with 50 mL of ammonium acetate. Samples were centrifuged for 30 minutes. K and Na were then determined by a direct reading of atomic emission, and Ca and Mg were determined using atomic absorption.

Statistical Analysis

I tested each soil characteristic with a generalized linear mixed model design, using maximum likelihood estimates. Depth, phase, and depth x phase interactions were fixed effects in each model (Zar 1999, Littell *et al.* 2006). For all models, I specified site, sample area (transect for soil moisture and bulk density, or consolidated sample for chemical analyses) within site, and depth within sample area as random blocking terms. Soil moisture, pH, organic matter, total N, Al, Ca, and Mg were natural log-transformed to approximate a normal distribution; other variables were tested without transformation. Post-hoc comparisons were determined significant differences by least squared means. All analyses were conducted in SAS 9.2, and I used $\alpha = 0.05$ for all analyses.

Results

Neither soil moisture nor bulk density differed by depth, phase, or depth x phase (Table 3.1). Mean bulk density across all treatments was 1.4 (±0.01) g/cm^3; mean moisture content was 12.1 (±0.69) %. pH significantly differed by depth x phase (Table 3.2; Fig. 3.1): at the shallow depth, pH was similar across phases, but for mid depth, pH was significantly lower in OF than G ($p = 0.0205$), and the deep samples exhibited lower pH in OF and PH compared to G ($p = 0.0401$ and 0.0062). Organic matter only differed by depth (decreasing with soil depth), but not phase or depth x phase (Fig. 3.2).

Total N also only differed by depth, and similar to organic matter, total N decreased with soil depth (Fig. 3.2). Al differed by all three factors: depth, phase, and depth x phase. By depth x phase, Al content was highest was in OF at the shallow depth, which was similar to levels only in YF; at mid-depth, high Al levels were also similar in OF and YF; at the deep depth, high Al value in OF was similar only to MF (Fig. 3.1). Al increased with increasing depth (Fig. 3.2), and by phase Al was highest in OF (Fig. 3.3). For Ca, I also found differences in depth x phase (Fig. 3.1); however, significant differences in least squared means revealed only differences between depths within the same phase. Similarly, while Mg did not differ between phases, there was a significant depth x phase interaction, but most of the depth x phase differences for Mg were between different depths within the same phase (Fig. 3.1). Mg also differed by depth: Mg was higher at the shallow depth compared to mid- and deep depths (Fig. 3.2). K also differed by depth, and similar to Mg, it was highest at the shallow depth (Fig. 3.2). Na only differed by depth x phase (Fig. 3.1), and similar to Ca, significant differences revealed only differences between depths within the same phase.

Discussion

Soil moisture

Surprisingly, I did not find differences in soil moisture related to depth or agroforestry management phase. Typically, soil moisture is lower in forests than grasslands (Fu *et al.* 2000), and decreases with stand age (Farley *et al.* 2005). This decrease in soil moisture in forests compared to grasslands may be due to differences in root distribution: grasslands dominated by herbaceous plants have denser root distribution in shallow soil, while woody plants (forests) access moisture from deeper layers (Walter 1971, Walter 1973).

However, from their paired catchment study in Uruguay, von Stackelberg et al. (2007) concluded that afforestation decreases runoff and peak flows during storms, but there was no reduction in base flow (Chescheir *et al.* 2008). Soil samples from my research were collected after short rains following an extended dry period; the soil moisture content I calculated (12.1%) is much lower than typical values for soil moisture in temperate grasslands, which are closer to 30% or higher (Knapp *et al.* 2002, Kisselle *et al.* 2002, Munhoz *et al.* 2008). Because I only collected soil moisture once, instead of throughout an extended period of time, I am limited in making conclusions about moisture patterns and the impact of afforestation.

Soil chemistry

In general, I did not find significant changes in soil chemistry or organic matter related to afforestation. Typically, afforestation of native grasslands decreases soil organic matter and total N, as well as lowering general fertility as other elements decrease in forest soils (Parfitt *et al.* 1997, Saviozzi *et al.* 2001). However, the long history of grazing in the Campos grasslands may have altered this expected result. Grazing may have increased organic matter concentrations in the Campos grasslands to levels expected with afforestation by increasing vegetation turnover (Franzluebbers *et al.* 2000). Additionally, grazed grasslands may exhibit low concentrations of macronutrients (particularly Mg, Na, and P), usually due to erosion of soil exposed by patchy removal of vegetation, resulting in areas of already low fertility before sites were afforested (Neff *et al.* 2005).

Although a substantial litter layer accumulates under the forest canopy, no corresponding increase in organic matter levels in the shallow soil samples was observed with agroforestry management phase. Likely, the dense fine root network associated with grassland vegetation and the typically high turnover rate of these roots contribute substantial amounts of organic matter to the soil, similar to inputs from litter and roots in forest phases (Brown & Lugo 1990, Guo & Gifford 2002).

The only nutrient that varied with phase was aluminum; similar to other research, Al was greatest in OF, when trees were most developed (Hughes *et al.* 1994, Giddens *et al.* 1997). However, the increase in soil aluminum is usually associated with the acidification of soil when grasslands are converted to forests (Adams *et al.* 2001), and although I did not find a significant change in pH associated with agroforestry management, there is a suggestion of a decreasing trend in soil pH across agroforestry management phases.

Even with fertilization in YF, before trees are planted, I did not see significant changes in soil characteristics (particularly total N or K); fertilization may have caused temporary changes in soil N that I did not detect in my sampling six months following planting (Merino *et al.* 2003, Laclau *et al.* 2005). Most of the potential change in soil fertility likely happens early in forest development, when trees are relying on nutrient uptake from existing resources in the soil; when trees are more developed, they rely on nutrient cycling through the system (Laclau *et al.* 2003). It is possible that my chronosequence phases do not adequately capture this potential short-term change in nutrient uptake.

Long-term changes in plantation forests

My research examined soil characteristics only through a first rotation. Some research in afforested systems has suggested that soil properties through the first rotation are similar to conditions during the previous land use (Binkley *et al.* 2004). It may require more time than one rotation for the legacy of the previous land use to be supplanted by soils characteristics associated with forest plantations. Major changes in soil nutrition as a consequence of land use change may be slow; the full impact of afforestation in the Campos may not be understood immediately (Jackson *et al.* 2000). For example, increases in soil carbon storage expected when open lands are converted to forests, may not happen for several more decades or after several more rotations (Attiwill 1994a, Paul *et al.* 2002). This factor may be especially relevant to eucalyptus forests that are harvested, on average, after 15 years (J.P. Posse, Weyerhaeuser Uruguay, pers. comm.). Some nutrients, like K, Ca, and Mg will likely increase after multiple rotations as residues from previous rotations left onsite to decompose are incorporated into the soil (Mendham *et al.* 2003). Additionally, loss of soil microbes associated with long-term forest presence will likely decrease soil fertility further (Berthrong *et al.* 2009).

While I provide some understanding of soil dynamics through a first rotation of grasslands afforested with eucalyptus, more research will be required to fully understand the impacts of afforestation. Because of the high variation in most soil variables, I suggest that a greater sample size may produce more conclusive results on effects of afforestation on grazed grasslands. While some of my results suggest a trend across agroforestry management phases, the high variability from my relatively small sample size resulted in mostly insignificant effects. In addition to a greater sample size, a more intensive sampling effort, including greater emphasis on short-term changes in soils characteristics particularly after planting and fertilization should be conducted. Other afforested areas in the Campos ecoregion are planted with *Pinus* sp., and their affects on soil characteristics may differ from *Eucalyptus* sp. (Scott & Lesch 1997, Huang *et al.* 2011). Also, I only studied the first rotation of these plantations, but I anticipate further changes as these sites are subjected to multiple rotations (Sharda *et al.* 1998). Because soils provide the structure and nutrition for vegetation, understanding the effects of afforestation on soils is critical for fully understanding the effects of afforestation on vegetation biodiversity (Kimmins 1997, Dupouey *et al.* 2002), which is increasingly important as afforestation continues and increases in distribution across the Campos.

Acknowledgements

I thank Gustavo Echevaleta and Gerardo Osorio for field sampling; Luciana Ingaramo, Juliana Ivanchenko, and Juan Pedro Posse for logistical help; Enrique Perez Gomar, María Bemhaja, and the INIA lab in Tacuarembó, Uruguay for soil sampling advice as well as laboratory testing; and Steve Duke for guidance on statistical techniques. Funding was provided by INIA (Instituto Nacional de Investigación Agropecuaria) and Weyerhaeuser Global Timberlands Research.

Table 3.1. Mixed model results for soil moisture and bulk density. No significant effects were detected.

		Num DF	Den DF	F Value	*p*-value
Moisture (%)	Depth	2	4	3.25	0.1452
	Phase	4	14	1.60	0.2293
	Depth x Phase	8	98	1.79	0.0889
Bulk density (g/cm^3)	Depth	2	4	0.06	0.9458
	Phase	4	14	1.18	0.3619
	Depth x Phase	8	100	1.35	0.2282

Table 3.2. Mixed model results for soil pH, organic matter, and chemicals (N, Al, Ca, Mg, K, and Na). Significant results from mixed model analysis are bolded.

		Num DF	Den DF	F Value	*p*-value
pH (H_20)	Depth	2	2	14.57	0.0538
	Phase	4	14	1.44	0.2737
	Depth x Phase	8	64	5.99	**<0.0001**
Organic matter (%C)	Depth	2	2	99.51	**0.0099**
	Phase	4	14	1.55	0.2427
	Depth x Phase	8	64	1.83	0.0881
Total N (%)	Depth	2	2	30.47	**0.0318**
	Phase	4	14	1.78	0.1898
	Depth x Phase	8	64	2.03	0.0567
Al (meq/100g)	Depth	2	2	55.74	**0.0176**
	Phase	4	14	4.00	**0.0228**
	Depth x Phase	8	64	2.96	**0.0071**
Ca (meq/100g)	Depth	2	2	9.06	0.0994
	Phase	4	14	0.74	0.5779
	Depth x Phase	8	64	3.05	**0.0057**
Mg (meq/100g)	Depth	2	2	30.93	**0.0313**
	Phase	4	14	1.23	0.3435
	Depth x Phase	8	64	7.11	**<0.0001**
K (meq/100g)	Depth	2	2	45.47	**0.0215**
	Phase	4	14	0.48	0.7481
	Depth x Phase	8	64	2.08	0.0508
Na (meq/100g)	Depth	2	2	1.85	0.3515
	Phase	4	14	0.31	0.8656
	Depth x Phase	8	64	2.41	**0.0242**

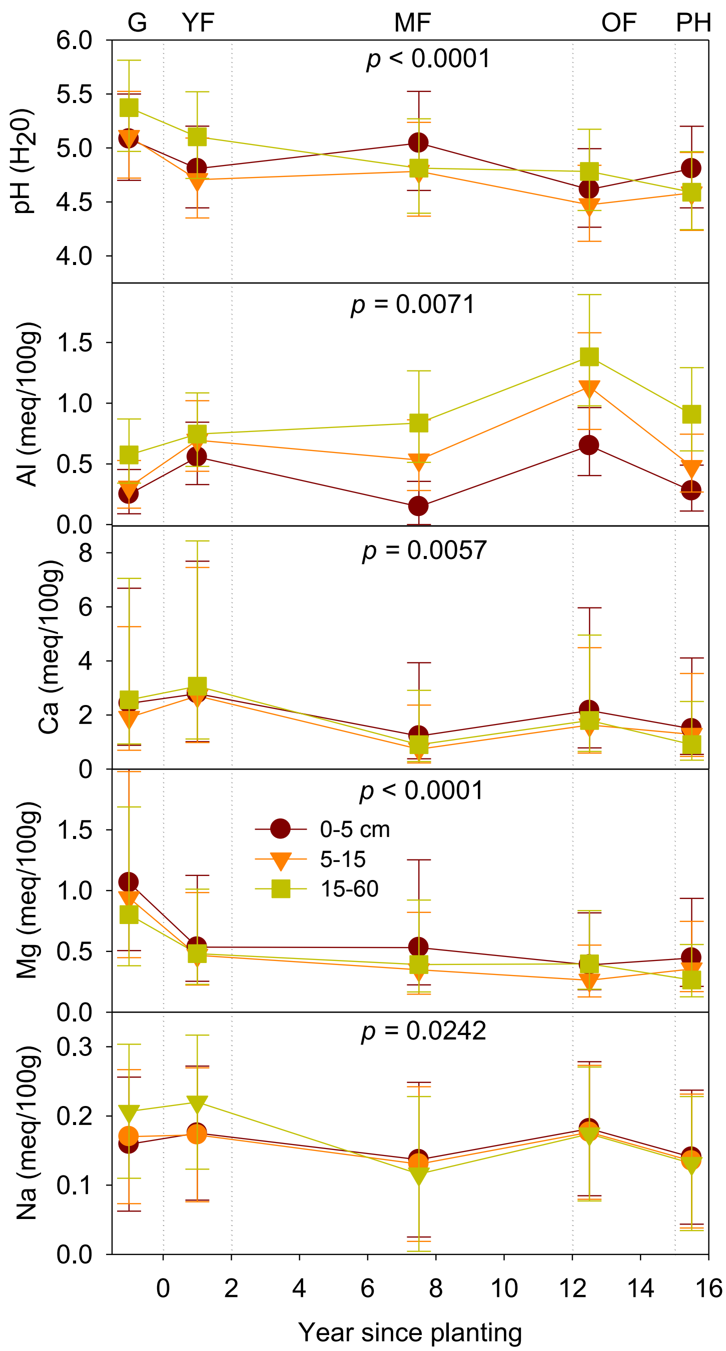

Figure 3.1. Soil pH and concentrations of Al, Ca, Mg, and Na in soil by depth across agroforestry management phases: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH) Results were determined from generalized linear mixed models. Data points represent means with 95% confidence intervals.

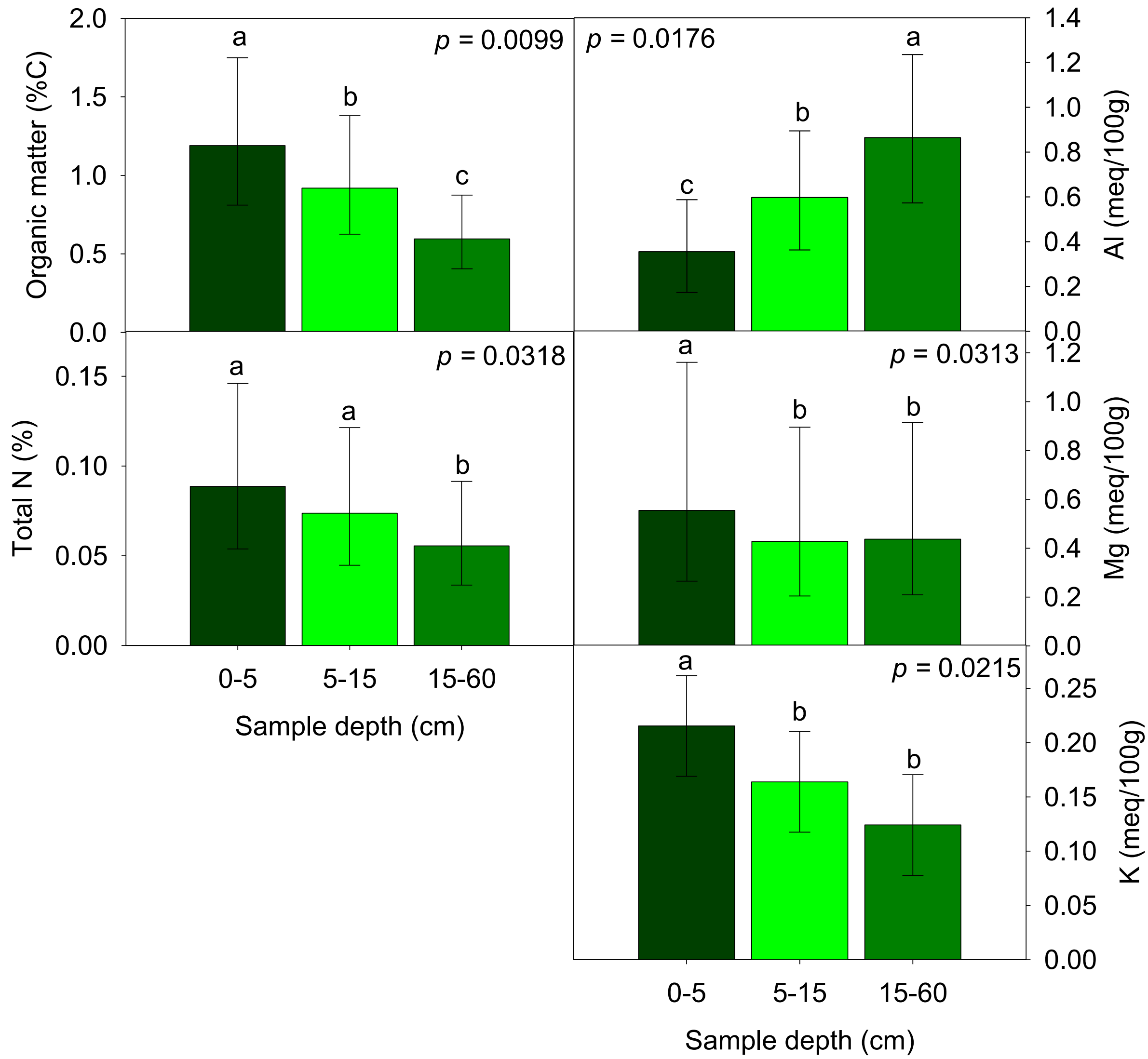

Figure 3.2. Differences in soil depth for organic matter, total N, Al, Mg, and K, determined from generalized linear mixed model results. Bars represent means with 95% confidence intervals; for each response variable, differences in least squared means are denoted by lowercase letters.

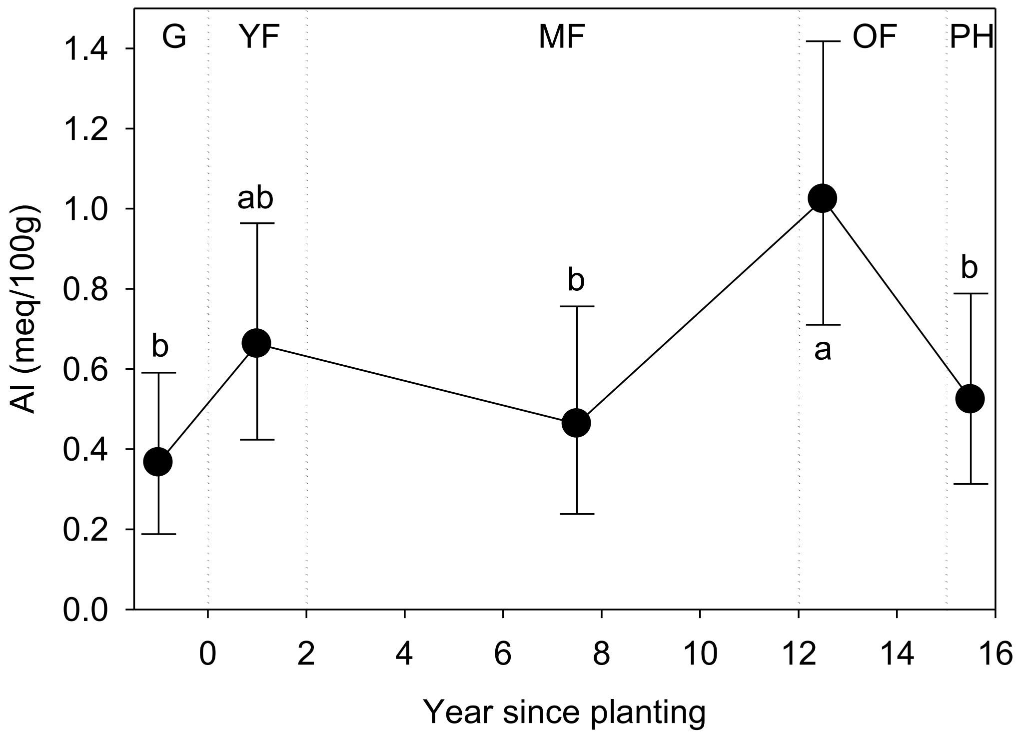

Figure 3.3. Differences in Al content by agroforestry management phase: Grassland (G), Young Forest (YF), Mid-stage Forest (MF), Old Forest (OF), and Post-Harvest (PH).Results were determined from a generalized linear mixed model. Data points represent means with 95% confidence intervals. Differences in least squared means are denoted by lowercase letters.

Chapter 4
Afforestation and grazing effects on Uruguayan grassland vegetation at multiple spatiotemporal scales

Abstract

The grasslands of the Campos region of southern South America have long been impacted by livestock grazing, and more recently, by widespread afforestation. I studied the influence of spatiotemporal scale on the response of vegetation to afforestation and livestock grazing in the Campos grasslands. Many ecological phenomena are scale-dependent, and these relationships can change among habitats and with disturbance. My study had two objectives: 1) compare species area curves to determine at which scales habitat type (forest and grassland), grazing management (grazed or ungrazed), and season (sample date) affect understory vegetation, and 2) evaluate habitat, management, and seasonal effects on species richness (proportion of richness by nativity and life form traits). I calculated species richness in modified-Whittaker nested plots of varying sizes (1, 4, 9, 36, and 144 m^2) for each treatment type (grazed forest, ungrazed forest, grazed grassland, and ungrazed grassland) at 5 sites. I tested the effects of habitat, management, and sample date on intercepts (small-scale richness) and slopes (rate of species accumulation) of species area curves. I also tested the effects of habitat, management, sample date, and interactions on total species richness and proportion of native species, as well as proportion by life form trait. Species area curves revealed differences in richness at small-scales between habitats (grasslands had more species than forests), but a greater rate of species accumulation in forests compared to grasslands led to little difference in richness at larger scales. In addition, there was also a greater rate of species accumulation in ungrazed compared to grazed treatments. There was a significant interactive effect on total species richness: in grazed habitats, richness varied by season (richness was greater in autumn in forests, but spring in grasslands), but in ungrazed habitats, richness varied more with time since exclosures were established (ungrazed grasslands decreased in species richness, but forests increased in richness). My results illustrate that the response of plant species richness to disturbances varies by the nature of the disturbance mechanism, and that multiple disturbances have complex interactive effects on vegetation. Examining vegetation response at multiple spatiotemporal scales was necessary to fully appreciate the complex nature of the impacts of these land-uses have on plant community composition.

Introduction

Many factors control the composition and richness of plant communities. Climate, soil conditions, biogeographical factors and many other elements act in concert to determine the mix of species that occupy a landscape (Cornell & Lawton 1992, Chapin *et al.* 1995, van der Heijden *et al.* 2008). One important determinant of plant community characteristics is disturbance. Catastrophic natural disturbance phenomena, like fires or flooding, alter soil characteristics and nutrient availability, impact the existing vegetation, and influence availability of species to re-colonize disturbed sites (Hobbs & Huenneke 1992, Collins *et al.* 1998). More chronic disturbance mechanisms, like grazing, can also have a profound effect on plant communities (Chaneton & Facelli 1991, Milchunas & Lauenroth 1993, Landsberg *et al.* 2002, Dorrough *et al.* 2007, Odion & Sarr 2007, Marini *et al.* 2009). As a result, disturbances can be of primary importance in structuring plant communities in many ecosystems (Hobbs & Huenneke 1992).

Vegetation responds rapidly to many types of disturbances. Li et al. (2007b) found that the vegetation community responded to changes in grazing, fire regimes and soil disturbance within the first several months following disturbance. Other research has concluded that vegetation response to some disturbances may take a longer time and the nature of the response may be influenced by the historical disturbance regimes to which the community was exposed (Fidelis *et al.* 2012).

Many ecological patterns and processes are scale-dependent (Crawley & Harral 2001, Briske *et al.* 2003, Kallimanis *et al.* 2008). Species-area relationships are a well-established manifestation of this phenomenon (see review in Palmer & White 1994), and as sample area increases, species richness also increases (Colwell & Coddington 1994). The rate at which species richness increases with scale varies by habitat and with disturbance (Denslow 1995, Lande *et al.* 2000, Keeley *et al.* 2003, He *et al.* 2006). In addition, variation in species richness characterized at small spatial scales is often high but decreases as the size of sample units increases (Fuhlendorf & Smeins 1996).

The grasslands of the Campos region of southern South America have been impacted by grazing, primarily by cattle and sheep, for several centuries, and more recently, widespread afforestation has occurred on these grasslands (Carrere & Lohmann 1996, Geary 2001). Afforestation, the practice of actively planting forests where none existed in recent history, has been widespread in Europe since the 20th century, but has only been common practice elsewhere within the last 30 years (Mather 1993). In Uruguay, where this study was conducted, 23% of the land base (approximately 4 million ha) has been designated for afforestation (Fig. 4.1); of this, nearly 1 million ha have already been planted (Uruguay XXI 2010). Landscape-scale forest habitat was not common prior to recent afforestation (e.g., Darwin 2009): native forests cover less than 4% of Uruguay's total land base (Geary 2001), mostly located along rivers and streams.

Grazing has been shown to affect richness at local and regional scales (Olff & Ritchie 1998, Fuhlendorf & Smeins 1999), although the effects tend to be more pronounced at local scales (Coughenour 1991, Beever *et al.* 2008). Low or moderate intensity grazing can cause an increase in richness at local scales but has been associated with decreased richness at coarse scales (Landsberg *et al.* 2002). However, high grazing intensity tends to decrease richness at all spatial scales (Chaneton & Facelli 1991, Dorrough *et al.* 2007, Odion & Sarr 2007, Marini *et al.* 2009). Grazing also lowers the slope of species-accumulation curves, as the rate of increasing species with increasing area is decreased with grazing (Spiegelberger *et al.* 2006).

Less is known about the effects of afforestation on plant diversity in the Campos region but there is evidence that this practice can affect species richness (Duarte *et al.* 2006). Some studies suggest that at small scales, species richness and diversity are greater in grasslands than forests, but at large scales there is no difference (del Pilar Clavijo *et al.* 2005, Buscardo *et al.* 2008). However, other research has indicated that the disturbed soil and variability in light environments associated with forest establishment may actually encourage greater diversity of understory species, grasses in particular (Naumburg & DeWald 1999).

Understanding the effects of grazing and afforestation is made more difficult in systems like the Campos grasslands that undergo seasonal shifts in vegetation. In general, more species are present in these grasslands during the cool season (autumn) than the warm season (spring) (Texeira & Altesor 2009), but there may be more spatial homogeneity in community composition in spring (Kohler *et al.* 2004). Seasonal plant responses to grazing is variable (Pollock *et al.* 2007), but seasonal changes may more strongly influence vegetation than grazing in some ecosystems (Kohler *et al.* 2004). There is no information on the effect of afforestation on seasonal changes in plant communities in this region.

Previous research on the effect of scale on plant species richness has evaluated scales of up to several ha, but the strongest scale dependence occurs within the 1 m^2 to 100 m^2 range (He *et al.* 2006). While it is known that different habitat types have distinct scale-dependent relationships (He *et al.* 2006), much less is known about how these scale-dependent relationships are influenced by multiple management activities, like grazing and afforestation. My study examines the response of plant species richness to grazing and afforestation at multiple spatial scales, by addressing two specific objectives:

1. Calculate species area curves to estimate how habitat type (forest or grassland), management (grazed or ungrazed), and sample date (season and time since exclosure) affect vegetation richness at multiple spatial scales, and

2. Evaluate habitat (forest or grassland), management (grazed or ungrazed) and sample date (season and time since exclosure) effects on species richness (total richness and proportion of richness by origin and life form trait).

Methods

Study Area

Research was conducted in the Tacuarembó region of northern Uruguay (Fig. 4.1). Average annual precipitation (1979-2010) is approximately 1460 mm, with the greatest monthly precipitation (165 mm) occurring in April, and the lowest monthly average precipitation occurring in August (80 mm) (INIA 2011). Rainfall can be highly variable, and droughts can occur throughout the year (Berretta *et al.* 2000), although there is no prolonged dry season (Overbeck *et al.* 2007). Mean annual temperature ranges from 16 to 19°C, with mean summer temperatures ranging from 22 to 27°C, and mean winter temperatures ranging from 13.5 to 16°C (Berretta *et al.* 2000, Pallares *et al.* 2005). The soils of the area are formed from loess deposits of unconsolidated silt and sand (Foth & Schafer 1980, Paruelo *et al.* 2007, Brady & Weil 2008). Soils are heterogeneous in the Campos, but in the study area, soils are generally fertile with mollisols dominant and alfisols in humid areas (Foth & Schafer 1980, Soriano *et al.* 1991).

Pinus species, *Eucalyptus globulus* and *E. grandis* are the most common tree species used in afforestation in Uruguay, typically planted as a monoculture. *Pinus* species monocultures are typically planted in lowlands and shallow valleys (J.P. Posse, Weyerhaeuser Uruguay, pers. comm.), since they are more frost-tolerant than *Eucalyptus* species, which tend to be planted on hill slopes. Within the afforested landscape, 34% of the land is not planted including lowland wet areas, firebreaks, fence rows, and sites that support native forests.

Field Methods

Data were collected in managed forest plantations in the northeastern region of the Tacuarembó department, Uruguay. Five study sites were selected, consisting of a 10-11 yr old (at the time of study establishment) *Pinus taeda* plantation and adjacent grassland (site photographs are provided in Appendix D). I measured several site characteristics within the plantations; mean values with standard errors are: 28.0 (±0.6) cm diameter, 523 (±43.15) trees per hectare, and 91.3 (±1.6) % canopy cover. In grasslands, canopy cover was 3.5 (±0.6) %. At each site, a 0.5 ha (50 × 100 m) exclosure was built across the forest grassland boundary in October 2009, with 0.25 ha exclosed area each of grassland and forest. An unfenced study area of the same dimension was established adjacent to each exclosure, also including grazed grassland and forest areas. Therefore, each of the five study sites included a 50 x 50 m treatment area of grazed forest plantation, ungrazed forest plantation, grazed grassland, and ungrazed grassland. Data were collected at the time when exclosures were built, and at six month intervals for two years (Spring 1 (0 months after exclosures were established), Autumn 1 (6 months after exclosures were established), Spring 2 (12 months after exclosures were established), and Autumn 2 (18 months after exclosures were established)).

Within each treatment area, a permanent plot was established, and modified Whittaker plot sampling method was employed. Each permanent plot was 12 × 12 m, and contained various combinations of plot sizes and sample sizes: (1 m^2 (n = 10), 4 m^2 (n=2), 9 m^2 (n=2), 36 m^2 (n=1), and 144 m^2 (n=1); Fig. 4.2). Within each plot, I recorded all species present using a modified cover class system: 1 = 0-1%, 2 = 1-5%, 3 = 5-25%, 4 = 25-50%, 5 = 50-75%, 5 = 75-95%, 6 = 95-100%. Species identifications proved challenging due to the dearth of botanical research conducted in this region, and as a result, several identifications could only be made to broader categories (family or growth form). Species were categorized into one of six potential life forms: (annual graminoid, perennial graminoid, annual herb, perennial herb, fern, or woody species) and nativity (native or introduced), based upon Zuloaga *et al.* (2008).

Analysis

I developed species area curves for each site for each season, habitat, and management combination (n=80) (Fig. 4.3), and calculated intercepts (small-scale species richness) and slopes (rates of species accumulation) for each curve. I then used a generalized linear mixed model to test differences in slopes and intercepts by sample date (0, 6, 12, or 18 months since exclosures were established), management (grazed or ungrazed), habitat (forest or grassland). I also included all two-way interactions, and sample date x management x habitat interaction. For both models, site and site x habitat x management were included as blocking terms and sample date was specified as a repeated measure, with an autoregressive structure. Results are displayed as hypothesis tests for the significance of each fixed effect and interaction (called a Type 3 Test). Post-hoc comparisons in significant differences were determined using least squared means. I set α = 0.05 for all analyses, and conducted all analyses in SAS 9.2/

I also calculated site-level total species richness and richness by origin and life form trait categories. Generalized linear mixed models were structured as described above. Total richness was count data, and therefore, was log-transformed as for a Poisson distribution. For origin and life form trait categories, data were tested as a proportion (number of species of each trait divided by total number of species) and were logit transformed as for a binomial distribution. There were too few annual graminoids to be analyzed fully, and the model would not converge when the three-way interaction was included; therefore, for annual graminoids, I excluded the three-way interaction from the final model.

Results

In total, I encountered 309 unique species; the number at individual study sites ranged from 192 to 217 species. In forests there were, on average, 239 species, while in grasslands there were 248 species. I was able to categorize 66% by life-form trait: of those, 52% were perennial herbs, 21% were perennial graminoids, 13% were annual herbs, 12% were woody species, 2% were annual graminoids, and 1% were ferns. Nativity status (native or introduced) was determined for 65% of species; of these, 95% were native species, and 5% (10 species) were introduced.

The intercepts of species area curves differed by sample date and habitat (Table 4.1, Fig. 4.4). Small-scale species richness was greater during the first three sample dates compared to the last, and grasslands had greater small scale-species richness than forests. In addition to these main effects, sample date x management was marginally significant: small-scale species richness during the last sample date in ungrazed areas was lower than all other sample dates and management combinations.

The slopes of species area curves differed by sample date, management, habitat and sample date x management (Table 4.1; Fig. 4.4). The last sample date (18 months after exclosures were established) had a faster rate of species accumulation than earlier sample dates, and the rate of species accumulation was greater in ungrazed than grazed treatments, and in forests than grasslands. Ungrazed treatments (grassland and forest, combined) 18 months after exclosures were established had a faster rate of accumulation than all other sample date x management combinations; all others were similar).

Total species richness differed by sample date x habitat, and sample date x management x habitat (Table 4.2). When examined by sample date x management x habitat interactions, there were several significant differences (Fig. 4.5). Total species richness in ungrazed forests was lowest during Spring 1, and was higher in Autumn 1 and Spring 2, suggesting some increase in richness following the exclusion of livestock. Species richness in grazed forests was generally higher in autumn than spring sample dates. In grazed grasslands, richness only differed between the second and third sample dates. For ungrazed grasslands, richness was highest in Spring 1, and then declined, indicating a decline in richness once cattle were excluded.

The proportion of native species differed by sample date and sample date x habitat (Table 4.3). In forests, the proportion of native species was higher in Autumn 1 and Spring 2 compared to Spring 1, and in grasslands, the proportion of native species in Spring 1 was lower than in Autumn 1, Spring 2, and Autumn 2 (Fig. 4.6); resulting in the main effect of sample date indicating the lowest proportion of native species richness during Spring 1.

Life-from traits differed by several main effects and one interactive tem (annual herbs differed by sample date x habitat). Differences by sample date were found for annual graminoids and herbs and perennial graminoids and herbs (Table 4.4, Fig. 4.7). The proportion of both perennial graminoids and herbs were lower during Spring 1 than subsequent sample dates. Conversely, the proportion of annual herbs was lower during Autumn 2. The proportion of annual graminoids was highest during Autumn 2 compared to all other sample dates. The proportion of perennial herbs, annual herbs, and ferns differed by habitat: the proportion of perennial herbs and ferns was higher in forests, but annual herbs constituted a higher proportion of the plant community in grasslands (Fig. 4.8). The proportion of woody species did not differ by any factor.

Discussion

Small-scale species richness and species accumulations

My finding that grasslands are more species rich than forests at small scales is similar to previous research that found that species richness is typically higher in open environments than forests (Alrababah *et al.* 2007, Buscardo *et al.* 2008, Bakker *et al.* 2010, Bremer & Farley 2010), likely due to consistently higher light regimes and higher levels of soil nitrogen, calcium, and organic carbon in grasslands (Spiegelberger *et al.* 2006, Buscardo *et al.* 2008). In addition, the dense litter layer under pine forests compared to grasslands likely results in a reduction of species richness (Wayman & North 2007).

This reduction in species richness in forests compared to grasslands is similar to my results in Chapter 2 (Understory vegetation change over an agroforestry management cycle in Uruguay): mid-stage eucalyptus forests (similar in age to pine forest in this chapter) showed the most drastic effects in understory vegetation, with reduced species richness and changes to composition. After forests were thinned, and later harvested, richness and composition were more similar to conditions found in grasslands.

In contrast, forests had a greater rate of species accumulations (greater slope) of species area curves than grasslands, and large-scale species richness was similar between habitats, suggesting that plants tend to be more patchily distributed under forest canopies than in grasslands, possibly due to heterogeneous conditions in light and soil fertility under the forest canopy (Tuomisto *et al.* 2003). While the removal of grazing did not have an overall effect on small-scale vegetation richness, there was a significantly higher rate of species accumulation in ungrazed compared to grazed areas, suggesting that vegetation in ungrazed environments is more patchily distributed than in grazed environments. This is somewhat surprising, since previous research has concluded that the direct (trampling and consumption) and indirect (nutrient distribution through urine and feces) effects of grazers leads to a heterogeneous environment in grazed habitats (Steinauer & Collins 1995). However, the Campos has been subject to long-term, relatively intense grazing, which may have led to relatively homogeneous conditions, reducing diversity and lowering rates of species accumulation (Spiegelberger *et al.* 2006).

Changes in total richness and proportion of life form traits

Differences in total richness by sample date may be attributed to both seasonal changes in vegetation as well as the amount of time that ungrazed areas were excluded from grazing. In the Campos grasslands, there is no dormancy period (Guerschman & Paruelo 2005), but vegetation varies seasonally, and in grazed habitats, I did see seasonal differences in vegetation: grazed forests tended to be more species rich in autumn than in spring, but in grazed grasslands, richness tended to be higher in spring. Previous research in the Campos has established that more species are present in these grasslands during the spring (Texeira & Altesor 2009). The higher richness in autumn in forests may be due to the fact that the cool-season vegetation found in autumn is more adaptable to shaded environments and lower temperatures found in forests.

In ungrazed habitats, the time since exclosure may have a more pronounced effect on richness than season. In ungrazed grasslands, species richness declined with time since exclosure. Other studies have shown that introducing grazing disturbance leads to increased species richness (Hartnett *et al.* 1996, Bokdam & Gleichman 2000); interestingly, this is similar to my findings, in a system where grazing disturbance is removed from an historically grazed environment. The reduction in species richness with cessation of grazing fits the intermediate disturbance hypothesis: grazing limits competitive species from becoming dominant, leading to increased species richness, so therefore, when grazing is removed, competitive species become dominant and total richness is reduced (Grime 1973, Connell 1978, Fox 1979, Altesor *et al.* 2005, Altesor *et al.* 2006).

Conversely, species richness increased in ungrazed forests during the middle sample dates of the study period (6-12 months following exclosure establishment). The increase in species richness in ungrazed forests is similar to other findings (Rummel 1951, Cooper 1960, Fleischner 1994). Unlike ungrazed grasslands, understory vegetation in forests tends to be relatively sparse and, as a result, exclusion of species by dominant competitors is less likely to occur. The elimination of grazing in the forests then enabled species sensitive to grazing pressure to increase.

I also found several differences in life form traits by habitat and management. Perennial herbs and ferns were proportionally more species rich in forests, while annual herbs were more proportionally rich in grasslands. This is somewhat surprising since annual species typically show a faster response to environmental change than perennial vegetation (Guo 2004). However, the accumulating litter layer in forests may inhibit seed germination and result in a decrease in annual species (Izhaki *et al.* 2000). The clonal spread of many perennial herbs may minimize the effect of the litter layer in the forest and enable access to resources from neighboring environments (Fahrig *et al.* 1994, Baum *et al.* 2009), thereby enabling relatively rapid recolonization of afforested sites and potentially contributing to greater richness of these species in forested habitats..

Long term potential change

The combination of grazing and afforestation in the Campos results in complex interactive impacts on vegetation, as plants respond to both disturbances (Chaneton & Facelli 1991, Milchunas & Lauenroth 1993, Landsberg *et al.* 2002, Dorrough *et al.* 2007, Odion & Sarr 2007, Marini *et al.* 2009). Interestingly, the disturbances differentially affected species richness, with grazing increasing species richness in grasslands compared to ungrazed grasslands, but grazing further decreasing richness in forests. While my study provides some understanding on these complexities, more work should be conducted to better understand longer-term effects of grazing and afforestation. Afforestation is a relatively new disturbance to the Campos ecoregion, and the exclusion of grazing was a change that was introduced only for the duration of this study.

Acknowledgements

I thank Cat Adams and Scott Batiuk for assistance with field sampling; Weyerhaeuser Uruguay staff for building exclosures at the study sites, and specifically Juliana Ingaramo, Luciana Ivanchenko, and Juan Pedro Posse for assistance with exclosure establishment and maintenance; Steve Duke for statistical guidance; María Bemhaja, Martin Juarena, and especially Eduardo Marchesi provided valuable contributions to species identifications and my understanding of grasslands in Uruguay. Funding was provided by Weyerhaeuser Global Timberlands Technology.

Table 4.1. Species area curves intercepts and slopes, as determined by Type 3 tests of fixed effects. Analyses were conducted using generalized linear mixed models that estimate maximum likelihoods. Effects in the model include sample date (Spring 1, Autumn 1, Spring 2, or Autumn 2), management (grazed or ungrazed), habitat (forest or grassland), and their two- and three-way interactions. Significant results are in bold.

		Num DF	Den DF	F Value	*P*-value
Intercept	Sample date	3	32	5.53	**0.0036**
	Management	1	12	1.27	0.2821
	Habitat	1	12	57.56	**<0.0001**
	Sample date x Management	3	32	2.75	0.0587
	Sample date x Habitat	3	32	2.12	0.1177
	Management x Habitat	1	12	0.86	0.3716
	Sample date x Management x Habitat	3	32	2.45	0.0818
Slope	Sample date	3	32	8.73	**0.0002**
	Management	1	12	5.54	**0.0365**
	Habitat	1	12	134.64	**<0.0001**
	Sample date x Management	3	32	4.37	**0.0109**
	Sample date x Habitat	3	32	1.59	0.2104
	Management x Habitat	1	12	1.08	0.3200
	Sample date x Management x Habitat	3	32	0.84	0.4819

Table 4.2.Generalized linear mixed model results for total species richness, as determined from Type 3 tests of fixed effects. Effects in the model include sample date (Spring 1, Autumn 1, Spring 2, or Autumn 2), management (grazed or ungrazed), habitat (forest or grassland), and their two- and three-way interactions. Significant results are in bold.

	Num DF	Den DF	F Value	*P*-value
Sample date	3	32	2.07	0.1237
Management	1	12	0.00	0.9896
Habitat	1	12	0.92	0.3570
Sample date x Management	3	32	1.31	0.2889
Sample date x Habitat	3	32	4.45	**0.0101**
Management x Habitat	1	12	2.07	0.1759
Sample date x Management x Habitat	3	32	6.39	**0.0016**

Table 4.3. Generalized linear mixed model results for proportion of native species, determined from Type 3 tests of fixed effects. Effects in the model include sample date (Spring 1, Autumn 1, Spring 2, or Autumn 2), management (grazed or ungrazed), habitat (forest or grassland), and their two-way interactions. Significant results are in bold.

	Num DF	Den DF	F Value	*P*-value
Sample date	3	32	6.03	**0.0022**
Management	1	12	3.05	0.1063
Habitat	1	12	0.14	0.7145
Sample date x Management	3	32	1.66	0.1962
Sample date x Habitat	3	32	3.02	**0.0441**
Management x Habitat	1	12	1.02	0.3332
Sample date x Management x Habitat	3	32	1.97	0.1389

Table 4.4. Generalized linear mixed model results for proportion of each life form trait, determined from Type 3 tests of fixed effects. Effects in the models include sample date (Spring 1, Autumn 1, Spring 2, or Autumn 2), management (grazed or ungrazed), habitat (forest or grassland), two-way interactions, and sample date x management x habitat. Significant results are in bold. The three-way interaction was not included in the model for annual graminoids as there were too few data to be analyzed fully, and the model would not converge when the three-way interaction was included.

		Num DF	Den DF	F Value	*P*-value
Annual graminoid	Sample date	3	34	4.26	**0.0117**
	Management	1	12	0.06	0.8147
	Habitat	1	12	1.27	0.2822
	Sample date x Management	3	34	0.19	0.9042
	Sample date x Habitat	3	34	1.26	0.3029
	Management x Habitat	1	12	3.01	0.1083
Annual herb	Sample date	3	32	4.25	**0.0124**
	Management	1	12	1.76	0.2096
	Habitat	1	12	40.74	**<0.0001**
	Sample date x Management	3	32	1.99	0.1353
	Sample date x Habitat	3	32	3.94	**0.0168**
	Management x Habitat	1	12	1.11	0.3122
	Sample date x Management x Habitat	3	32	0.96	0.4217
Fern	Sample date	3	32	0.09	0.9649
	Management	1	12	0.00	0.9461
	Habitat	1	12	6.25	**0.0279**
	Sample date x Management	3	32	0.19	0.9011
	Sample date x Habitat	3	32	0.10	0.9568
	Management x Habitat	1	12	0.00	0.9988
	Sample date x Management x Habitat	3	32	0.15	0.9269
Perennial graminoid	Sample date	3	32	8.02	**0.0004**
	Management	1	12	2.61	0.1320
	Habitat	1	12	0.96	0.3469
	Sample date x Management	3	32	0.17	0.9128
	Sample date x Habitat	3	32	2.10	0.1199
	Management x Habitat	1	12	0.01	0.9445
	Sample date x Management x Habitat	3	32	0.17	0.9185
Perennial herb	Sample date	3	32	5.23	**0.0047**
	Management	1	12	2.02	0.1803
	Habitat	1	12	13.92	**0.0029**
	Sample date x Management	3	32	0.14	0.9365
	Sample date x Habitat	3	32	1.20	0.3261
	Management x Habitat	1	12	0.41	0.5320
	Sample date x Management x Habitat	3	32	0.82	0.4900
Woody	Sample date	3	32	0.36	0.7828
	Management	1	12	4.48	**0.0560**
	Habitat	1	12	1.94	0.1888
	Sample date x Management	3	32	0.92	0.4407
	Sample date x Habitat	3	32	1.91	0.1480
	Management x Habitat	1	12	0.41	0.5335
	Sample date x Management x Habitat	3	32	0.39	0.7576

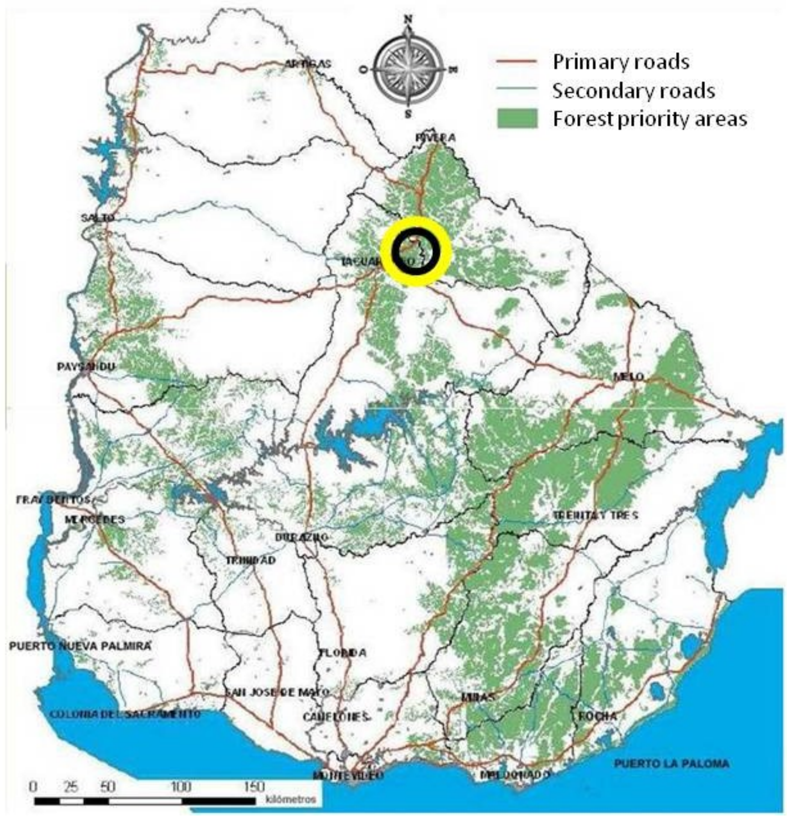

Figure 4.1. Location of exclosure study sites (with *Pinus taeda* and adjacent grassland) in north-central Uruguay, with areas designated as Forestry Priority (grasslands that have been or will be afforested) in green. Almost half (46%) of the planting has occurred in the North Central area of Uruguay, where my research occurred (5 study sites within the area indicated by the black and yellow circle) (Uruguay XXI 2010).

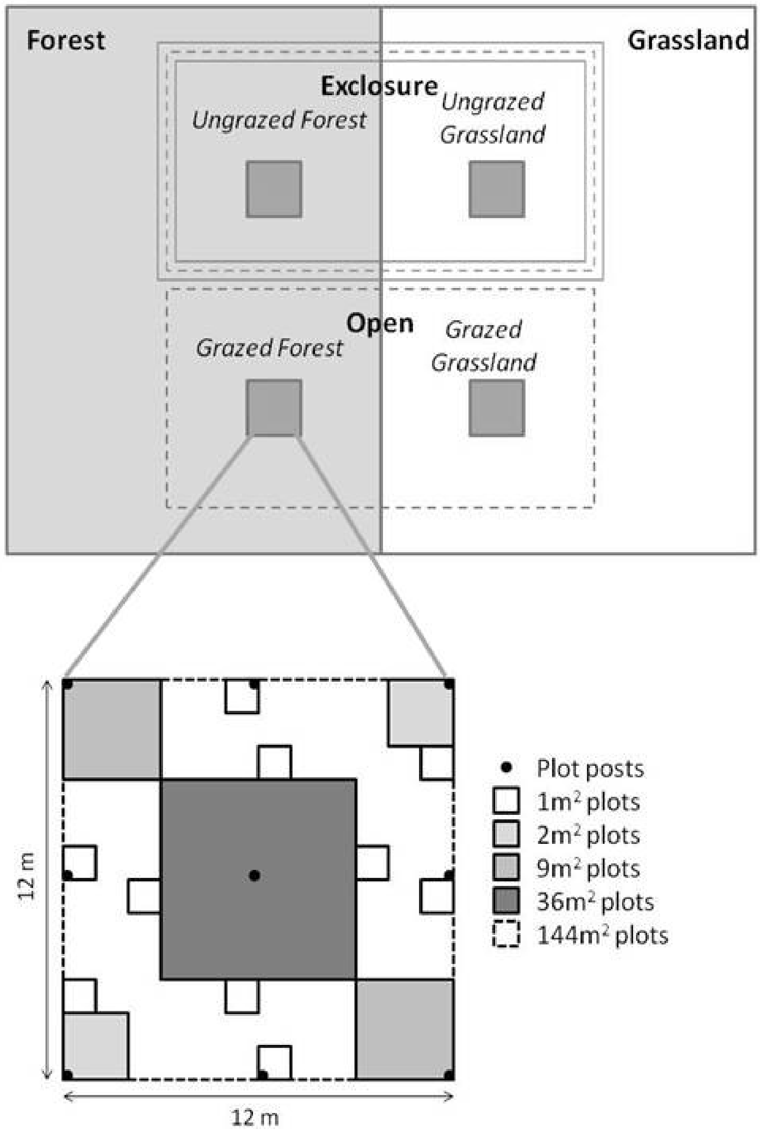

Figure 4.2. Study site layout and sampling design to collect vegetation richness at multiple spatial scales. A permanent nested plot was established within each treatment area at each site. I sampled using a modified Whitakker plot method. Plots were permanently marked at the corners and midpoints (black circles) with PVC pipe. Within each 12 x 12 m square, plots of different sizes were established systematically: 144 m^2 (*n*=1), 36 m^2 (*n*=1), 9 m^2 (*n*=2), 4 m^2 (*n*=2), and 1 m^2 (*n*=10). Species presence was recorded in each plot, with cover additionally collected in all 1 m^2 plots.

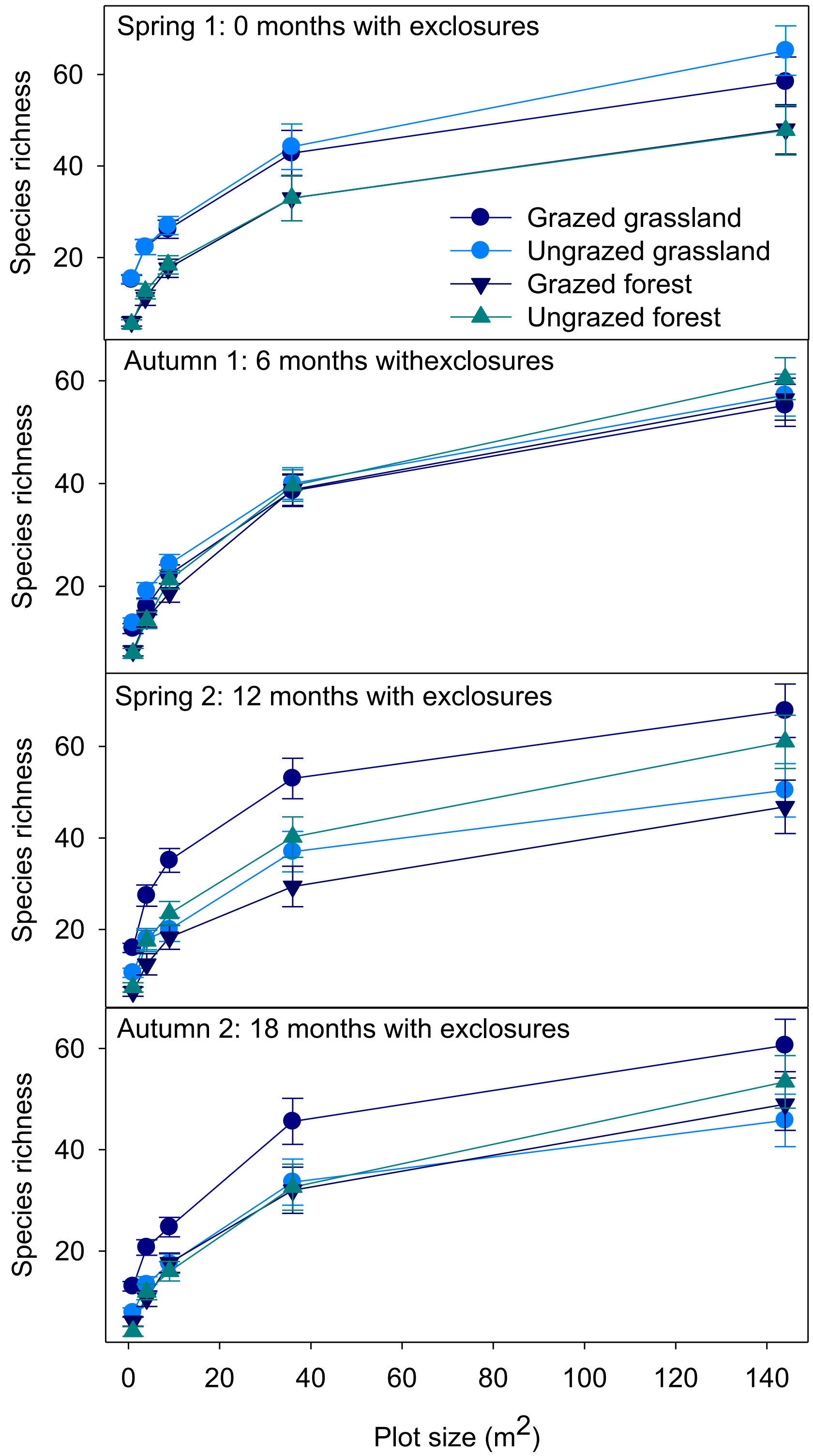

Figure 4.3. Species area curves by management x habitat combination for each sample date. Data points are means with standard errors.

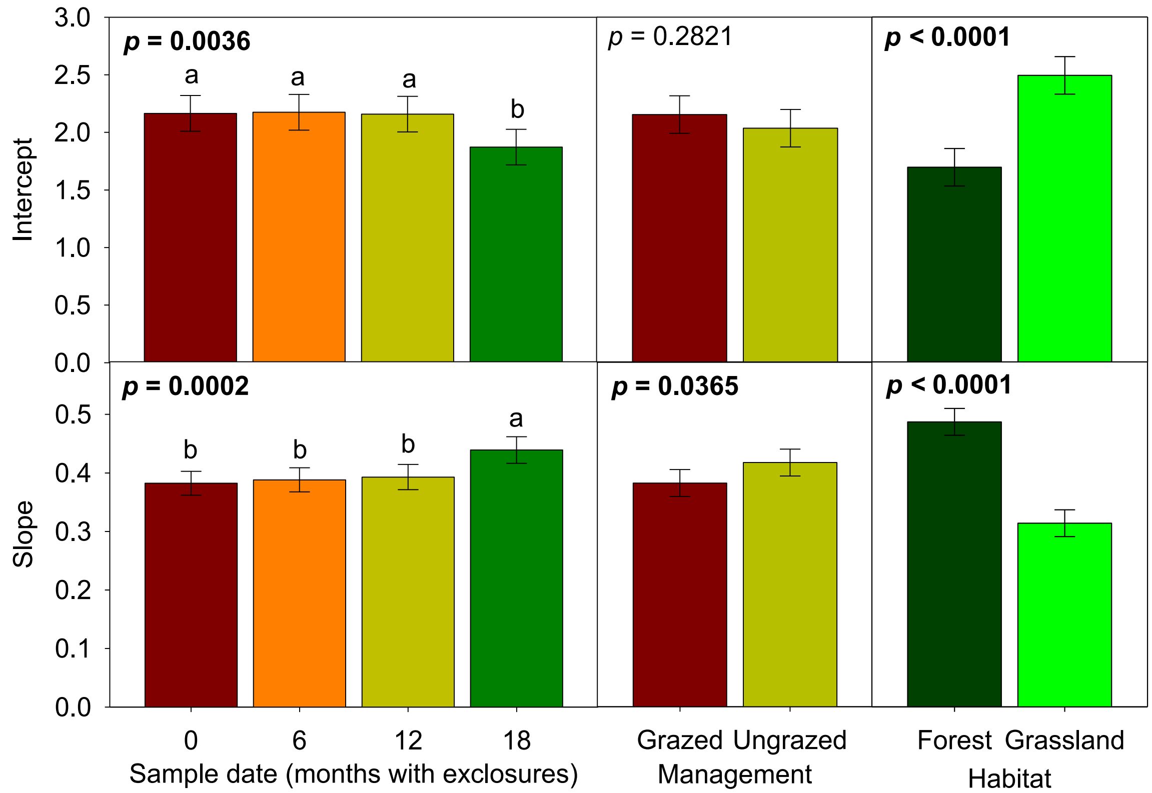

Figure 4.4. Species area curve intercept (top) and slope (bottom) values for sample date (left), management (center), and habitat (right). Bars are means with 95% confidence limits. Significant results have *p*-values in bold for season, significant differences in least squared means are denoted by lowercase letters.

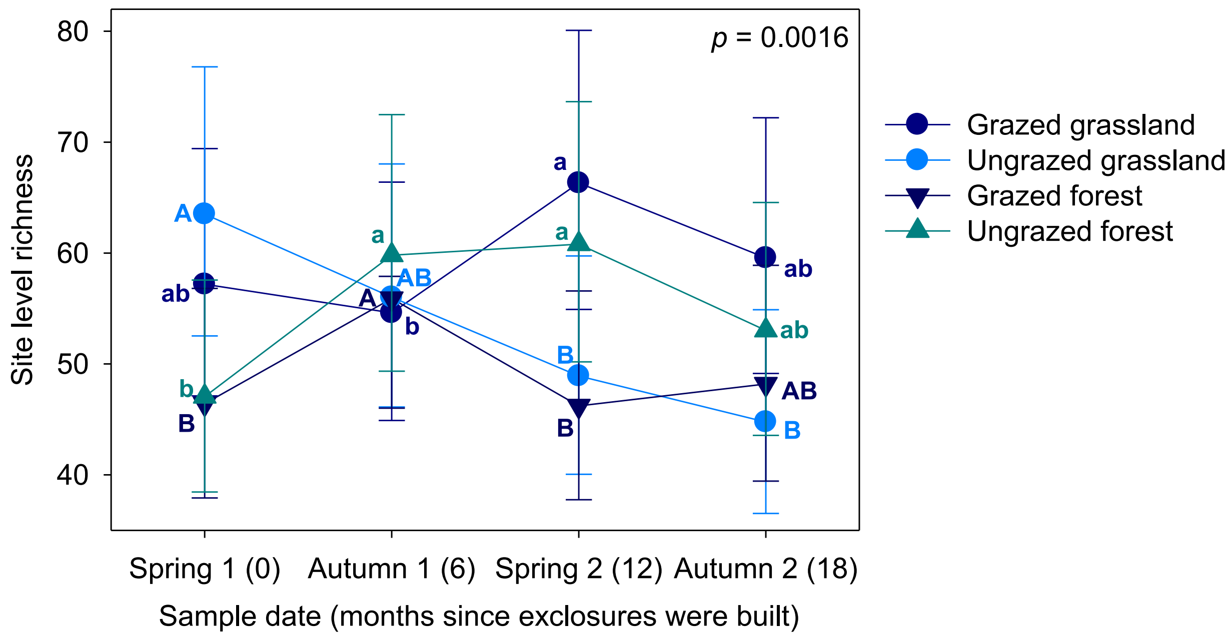

Figure 4.5. Total species richness (calculated at the site level) by sample date x management x habitat. Data points are means with 95% confidence limits. Differences in least squared means are denoted for each management x habitat combination: grazed grassland (lowercase, dark grey), ungrazed grassland (uppercase, dark grey), grazed forest (uppercase, black), and ungrazed forest (lowercase, light grey).

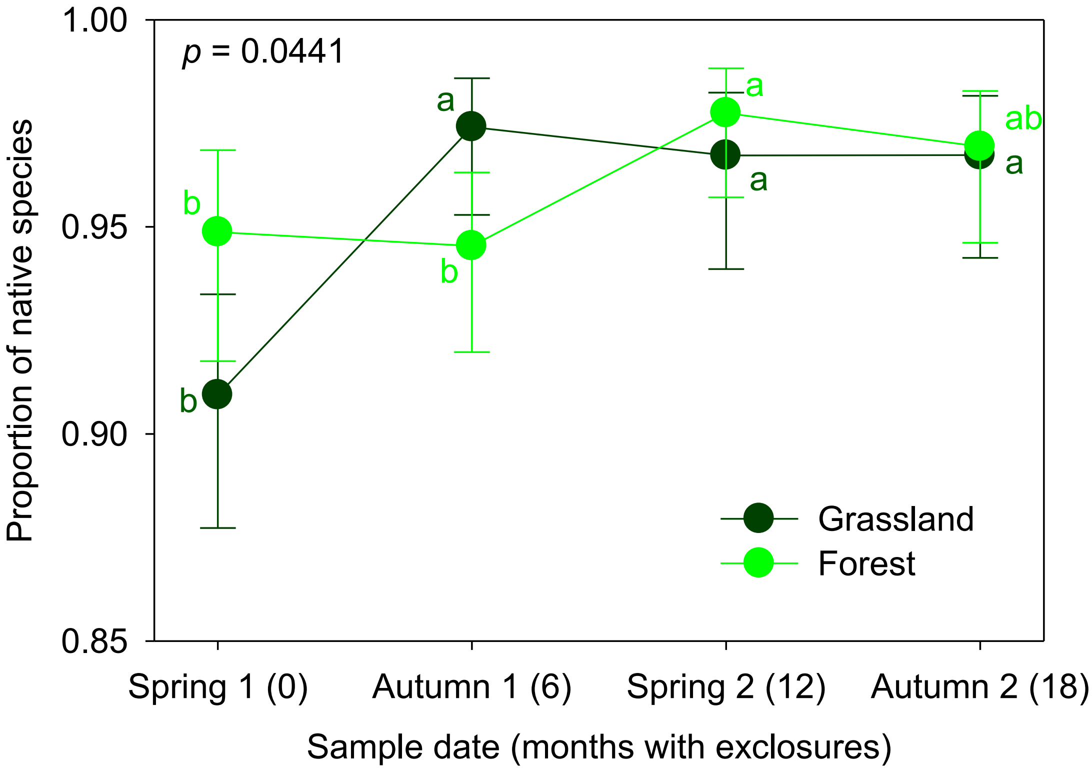

Figure 4.6. Proportion of native species by sample date x habitat. Data points are means with 95% confidence limits. For each habitat, differences in least squared means are denoted by lowercase letters.

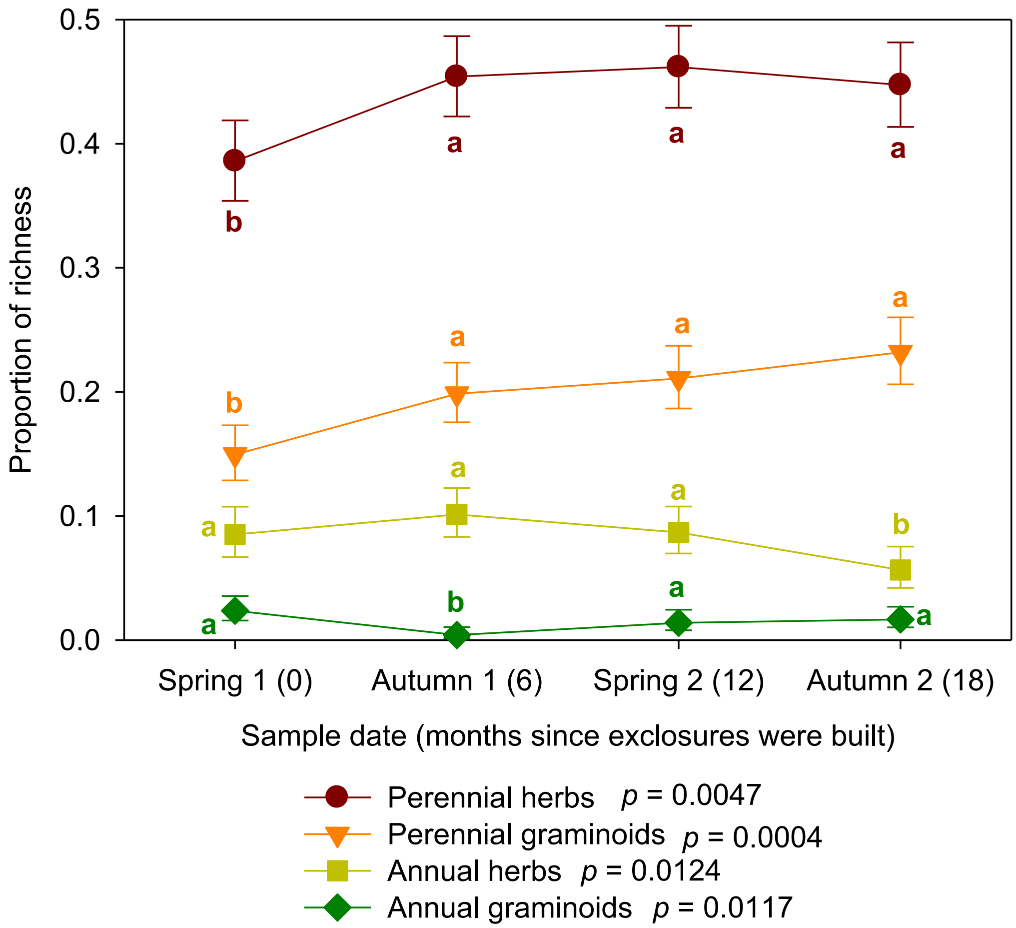

Figure 4.7. Proportion of annual and perennial herbs, and annual and perennial graminoids by sample date. Data points are means with 95% confidence limits; for each trait, differences in proportions by sample date are denoted in lowercase letters.

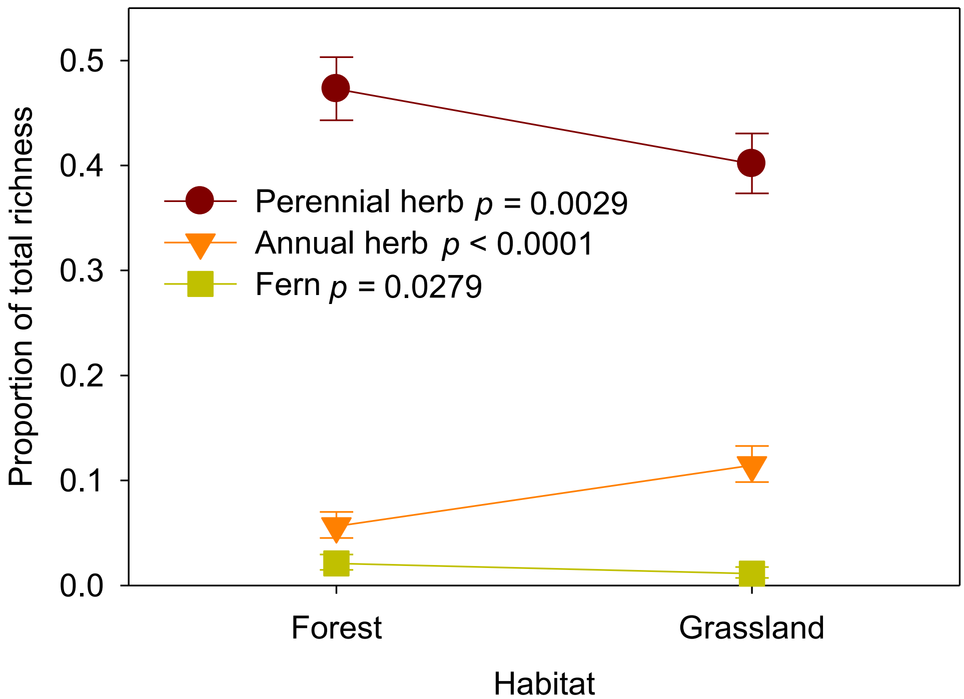

Figure 4.8. Proportion of annual and perennial herb and fern richness by habitat. Data points are means with 95% confidence limits.

Chapter 5
Conifer germination and establishment in plantations and adjacent grasslands of northern Uruguay

Abstract

Afforestation, the practice of planting trees where they did not occur recently, is a common practice around the globe. In southern South America, afforestation is occurring on grasslands that are adapted to long-term cattle grazing and agricultural cultivation. Exotic species are used for these plantations and potential spread of these trees into remaining grassland area is a growing concern. We quantified rates of encroachment by loblolly pine (*Pinus taeda*) in and adjacent to five plantations in northern Uruguay. Transects were placed in grazed areas and in areas where grazing was excluded. Areas were sampled semi-annually (spring and autumn) for two years to examine germination and establishment. Seedlings present in grasslands occurred only near the forest edge. In both spring and autumn, total seedling density differed by habitat (forests supported far more seedlings than grasslands) and year (in spring, more seedlings were present in year 1 than 2, but in autumn, more seedlings were present in year 2). My results suggest that loblolly pine encroachment into adjacent grasslands is unlikely. The mechanisms controlling encroachment differ: in ungrazed grasslands, the dense cover of herbaceous and grass species prevents establishment whereas in grazed grasslands, the intensive livestock grazing prevents tree establishment.

Introduction

Afforestation, the practice of actively planting forests where they did not occur recently, has been widespread in Europe since the 20th century but has only been common practice elsewhere within the last 30 years (Mather 1993, EFI 2000). Currently, seven percent (264 million ha) of forest area, globally, is afforested, and afforestation is increasing in importance as deforestation continues to be a global issue (FAO 2010). The Campos grasslands of South America are a focus of considerable afforestation. Historically, these areas contained little landscape-scale forest habitat (e.g., Darwin 2009): currently, native forests cover less than 4% of Uruguay's total land base (Geary 2001), mostly located along rivers and streams. Historically, the landscape of Uruguay consisted of grasslands that are adapted to long-term cattle grazing and agricultural cultivation (Carrere & Lohmann 1996, Geary 2001, Baldi *et al.* 2006, Baldi & Paruelo 2008, Vega *et al.* 2009).

In 1987, the government of Uruguay passed a forestry law to promote forest industry investment. As a result of this law, 23% of the land in Uruguay (approximately 4 million ha) has been designated for afforestation, of which nearly 1 million ha have already been planted (Uruguay XXI 2010). The large scale of the afforestation effort in Uruguay is decreasing the historic dominance of grasslands on the landscape (Dumig *et al.* 2008). Local scientists have expressed concern over conifer seedling encroachment from plantation forests into remaining grasslands, but to date, no research has been conducted on the occurrence, rate, or ecological implications of conifer encroachment (M. Bemhaja, INIA, pers. comm.; C. Sans Doble, Universidad de la Republica, pers. comm.). This concern is exacerbated by the fact that the fast-growing pioneer species used in plantation forests may be particularly successful invaders of adjacent grassland (Dzwonko & Loster 1997, Brockerhoff *et al.* 2008).

To encroach into adjacent area, seedlings must germinate and establish from a plantation (Buckley *et al.* 2005). Seed germination can be limited by moisture and light, and establishment (early survival) by low light conditions, such as under a forest canopy (Johnson & Young 1993, Buckley *et al.* 2005). Higher light in adjacent grasslands may provide a better environment for seedling establishment. However, in some areas the densities of seedlings and small saplings are similar between interior forest and grassland (Oosterhoorn & Kappelle 2000).

Livestock grazing was widespread before afforestation and continues in the plantations being established in Uruguay. Livestock can trample or graze tree seedlings. As a result, livestock are excluded from plantations until the planted trees reach sufficient size to be resistant to livestock damage (approximately two years after trees are planted). Livestock grazing also can alter the nature of competitive interactions between invading seedlings and grassland vegetation, particularly shrubs (Dunwiddie 1977, Ledgard 2001, Buckley *et al.* 2005, Boulant *et al.* 2009). In a comparison of various grazing intensities, Boulant et al. (2009) found that shrub cover hindered seedling invasion with no or low-intensity grazing, but facilitated it in systems with high-intensity grazing because shrubs provided seedlings with some protection from livestock damage.

Limited research on tree encroachment has been conducted in South America, and most previous research has focused on native or naturalized species (Van Auken 2000). Exotic species are used for most forest plantations in South America, and the potential for spread of these tree species into remaining grassland area, while of growing concern, is poorly understood. This study evaluated loblolly pine (*Pinus taeda*) seedling establishment and survival under plantations and in adjacent grasslands in the Uruguay Campos. As the Campos ecoregion covers a large area in Uruguay, northern Argentina, southern Brazil and Paraguay and loblolly pine is a commonly planted tree species, these results should have broad application. I have two specific objectives:

1. Examine seedling germination and establishment in plantations and in adjacent grasslands by sampling in spring and autumn, and

2. Determine effects of grazing pressure on encroachment.

Methods

Study Area

Research was conducted in the Tacuarembó region of northern Uruguay (for more details, see Chapter 4: Afforestation and grazing effects on Uruguayan grassland vegetation at multiple spatiotemporal scales). *Pinus taeda*, *P. elliottii*, *Eucalyptus globulus*, and *E. grandis* are the most common tree species used in afforestation in Uruguay. Typically, stands (which were previously swaths of grazed grassland) are planted as monocultures with *Pinus* species in lowlands and shallow valleys, since they are more frost-tolerant, and *Eucalyptus* species on hill slopes and ridges (J.P. Posse, Weyerhaeuser Uruguay, pers. comm.). Within the afforested landscape, about 35% of the land is not planted; the majority of unplanted land is in wet areas, firebreaks, fence rows, or at sites that currently support native forests.

Field Methods

Data were collected in five managed *Pinus taeda* plantations and adjacent grasslands. *P. taeda* stands were 10-11 yrs old at the time of study establishment, and have been producing seeds for 3-5 years (J.P. Posse, Weyerhaeuser UY, pers. comm.); mean diameter at breast height was 28 cm, and mean stand density was 523 trees per ha. At each site, a 0.5 ha (50 × 100 m) exclosure was built across the forest-grassland boundary in October 2009, with 0.25 ha exclosed area each of grassland and forest. A study area of the same dimensions was established adjacent to the exclosure in the grazed grassland and forest areas (Fig. 5.1).

Study sites were sampled immediately after exclosure construction in spring (October 2009) and at six month intervals until May 2011. At each of the four sampling dates, 1-m wide belt transects were randomly established, perpendicular to the forest-grassland edge, and extending 25 m into the forest stand and 25 m into the grassland within each management treatment (grazed or ungrazed). Seedling densities in spring provided a metric of germination, although they also included seedlings that may have germinated and established in prior years, while seedling densities in autumn provided a measure of establishment. Seedlings were generally young and of short stature, so they were recorded in two height classes (< 10 and > 10 cm) in 5-m intervals along each belt transect.

Analysis

Seedling density in grasslands was too low to permit analyses of distance from the forest-grassland edge, so densities were summed within each habitat and grazing treatment. A generalized linear mixed model (GLMM) was developed for total density, log-transforming the data to better approximate a normal distribution (Littell *et al.* 2006). Germination and establishment were distinguished by analyzing seasons (spring and autumn) separately. We tested the effects of management (grazed or ungrazed), habitat (forest or grassland), year (first or second) and two-way interactions on total seedling density. Sites were included in the models as a random blocking term.

GLMMs were also used to test for significant differences in the proportion of seedlings by size class. We tested the proportion of seedlings that were < 10 cm in height (number of small seedlings divided by total number of seedlings). This analysis had the same structure as total density analysis. Germination and establishment were distinguished by analyzing seasons separately.   The model included management, habitat, year, and all two-way interactions. Sites were included in the model as a random blocking term. I performed all analyses in SAS v.9.2, and we determined significance at $\alpha = 0.05$.

Results

Seedlings were found throughout forest plantations and in grasslands within 10 m of the plantation edge (Fig. 5.2). For both spring and autumn, seedling density significantly differed by habitat and year. For both seasons, seedling density was greater in forests than in grasslands: in spring, seedling density averaged 29689.31 trees per hectare (tph) in forests and 216.59 tph in grasslands , while in autumn, seedlings averaged 1422.50 tph in forests and 5.51 tph in grasslands (Fig. 5.3, Table 5.1). In spring, seedling density was significantly greater in year 1, but in autumn, seedling density was greater in year 2 (Fig. 5.4). Also in spring, seedling density significantly differed by habitat x year interaction ($p = 0.0299$): forests in year 1 and 2 were had the greatest seedling density (28765.19 and 30643.12 tph, respectively), and grasslands in year 1 had more seedlings than in year 2 (857.06 and 54.45 tph, respectively).

The proportion of small seedlings (< 10 cm tall) significantly differed by year for both spring and autumn (Fig. 5.5, Table 5.2). In spring, there was a greater proportion of small seedlings in year 1, but in autumn, there was a higher proportion of small seedlings in year 2. The proportion of small seedlings was not significantly affected by any other factor (although the proportion of small seedlings was marginally significant between habitats (p = 0.0510)).

Discussion

Conifer encroachment into grasslands is dependent on successful seedling germination and establishment in this habitat. My research found seedlings to be common under the forest canopy with a large seasonal shift; high seedling densities in spring months, followed by decreased density in autumn. Although we did not sample the same transects over time and therefore did not track the fate of individual seedlings, I attribute these seasonal differences in density to seedling mortality. This is similar to other studies showing peaks in seed rain and seedling emergence during wet months, followed by high mortality in summer months (Marques & Oliveira 2008, Parada & Lusk 2011). At my study sites, spring coincides with relatively high precipitation and warming temperatures, while autumn typically experiences high precipitation but cooling temperatures following relatively dry, warm summer conditions (INIA 2011). While this region does not experience a typical drought season during the year, irregular periods of drought can impact seedling survival (Marques & Oliveira 2008).

The few seedlings found in grassland areas were near the forest edge, which is similar to findings of other research (Copenheaver *et al.* 2004). Seeds of pines are mostly deposited directly underneath the existing tree crown, and seed supply is the most important factor for seedling recruitment (Dovciak *et al.* 2008, Boulant *et al.* 2009). Seedling establishment near the plantation also may be facilitated by changes to the local environment caused by the plantation, including shade from adjacent trees and sheltering from extreme temperatures (Fulco *et al.* 2001, Siemann & Rogers 2003, Coop & Givnish 2008). In addition, the thick pine needle little layer that develops under and adjacent to forest environments may actually positively affect seedling survival by inhibiting competition from further plant establishment (Richardson & Bond 1991). As remnant grasslands tend to be slightly lower in elevation than plantations, these areas can be subject to frost as well as higher moisture conditions (J.P. Posse, Weyerhaeuser Uruguay, pers. comm.), which may negatively affect seedling survival (see also Chapter 3: Soil characteristics through first-rotation Eucalyptus plantation cycle in Uruguay).

My results differ from several previous studies that found higher seedling densities in grasslands than in forests, mostly due to higher light conditions in open areas (Johnson & Young 1993, Dzwonko & Loster 1997, Buckley *et al.* 2005, Brockerhoff *et al.* 2008). Seedlings were largely absent from grasslands in this study. There are likely different mechanisms limiting tree seedling presence in grazed and ungrazed grasslands. In ungrazed grasslands, seedling establishment is likely limited by the dense cover of grasses and herbaceous species (Copenheaver *et al.* 2004). In grazed grasslands, seedlings are likely prevented from establishment through consumption and trampling. Grazing has been shown to reduce vegetation competition and thereby increase tree encroachment in some systems (Van Auken 2000). However, the intense grazing at our sites did not result in increased seedling density, a result also observed in other previous work (Le Bagousse-Pinguet *et al.* 2012).

While I found high seedling density in forests, the limited dispersal of pine seeds and high mortality of seedlings in grasslands caused by competition from surrounding vegetation in ungrazed settings or consumption and trampling in grazed areas, I suggest that pine encroachment into adjacent grasslands is unlikely at locations managed similar to our study sites. Therefore, conifer spread from plantations may not be a serious threat to remaining grasslands of the Campos ecoregion. However, I acknowledge the limitation that I only studied the first two years after exclosures were built, and it is possible I have not seen the full effects of grazing on conifer encroachment. Further, future work should be conducted to monitor the few surviving seedlings near the forest edge as they develop, as this could facilitate future seedling establishment. Also, other species of trees used in plantations in the Campos region (e.g., *Eucalyptus* species) are capable of vegetative reproduction (Parsons *et al.* 2006, Drake *et al.* 2009) and may pose a larger threat of encroachment on remaining grasslands. As these species are planted broadly throughout the region, a thorough assessment of their invasiveness will be required to fully appreciate the potential for trees to spread into grassland habitats in the Campos.

Acknowledgements

We thank Gustavo Echevaleta, Gerardo Osorio, and John Six for field assistance; Luciana Ingaramo, Juliana Ivanchenko, and Juan Pedro Posse for logistical help; and Steve Duke for guidance on statistical techniques. Funding was provided by Weyerhaeuser Global Timberlands Research.

Table 5.1. Total seedling density by management (grazed or ungrazed), habitat (forest or grassland), year (1 or 2), and two-way interactions, determined from type 3 tests of fixed effects from generalized linear mixed models that used maximum likelihood estimators. Significant effects are denoted by bolded *p*-values.

	Effect	Num DF	Den DF	F Value	*p*-value
Spring	Management	1	27	0.44	0.5149
	Habitat	1	27	64.10	**<0.0001**
	Year	1	27	4.80	**0.0373**
	Management x Habitat	1	27	0.19	0.6637
	Management x Year	1	27	0.00	0.9673
	Habitat x Year	1	27	5.26	**0.0299**
Autumn	Management	1	27	0.01	0.9137
	Habitat	1	27	32.45	**<0.0001**
	Year	1	27	8.65	**0.0066**
	Management x Habitat	1	27	0.25	0.6210
	Management x Year	1	27	1.34	0.2576
	Habitat x Year	1	27	0.73	0.3933

Table 5.2. Proportion of small seedlings by management (grazed or ungrazed), habitat (forest or grassland), year (1 or 2), and two-way interactions, determined from type 3 tests of fixed effects from generalized linear mixed models that used maximum likelihood estimators. Significant effects are denoted by bolded *p*-values.

	Effect	Num DF	Den DF	F Value	p-value
Spring	Management	1	27	0.90	0.3515
	Habitat	1	27	4.17	0.0510
	Year	1	27	4.48	**0.0437**
	Management x Habitat	1	27	0.24	0.6266
	Management x Year	1	27	0.00	0.9823
	Habitat x Year	1	27	1.55	0.2238
Autumn	Management	1	27	0.09	0.7667
	Habitat	1	27	1.58	0.2195
	Year	1	27	5.13	**0.0317**
	Management x Habitat	1	27	0.26	0.6150
	Management x Year	1	27	0.98	0.3317
	Habitat x Year	1	27	2.36	0.1365

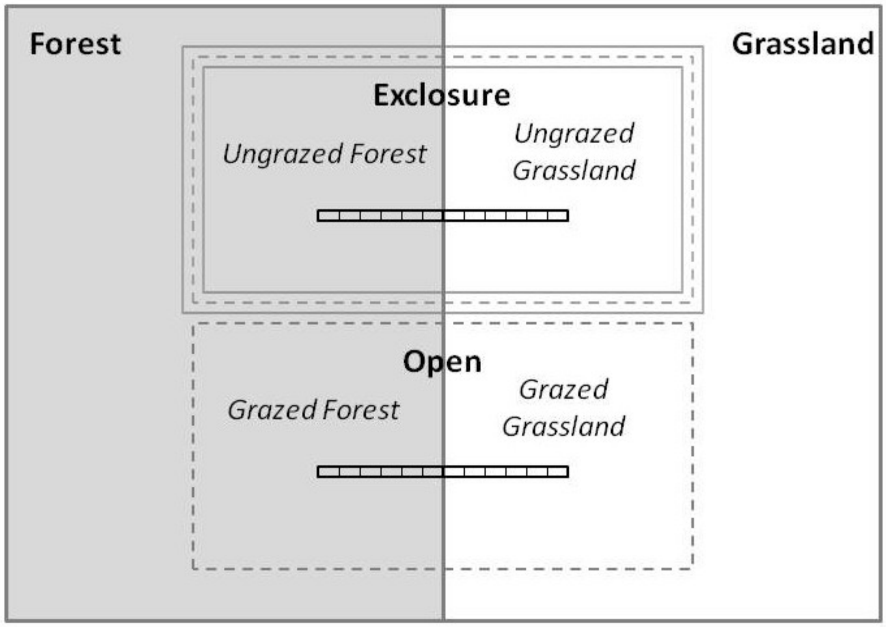

Figure 5.1. Study design employed to determine seedling density in exclosed and open areas at each study site. Each exclosure was 50 x 100 m. A transect (1 x 50 m, divided into 5 m segments) was randomly placed within each treatment area (inside exclosure and in adjacent open area) each sampling season.

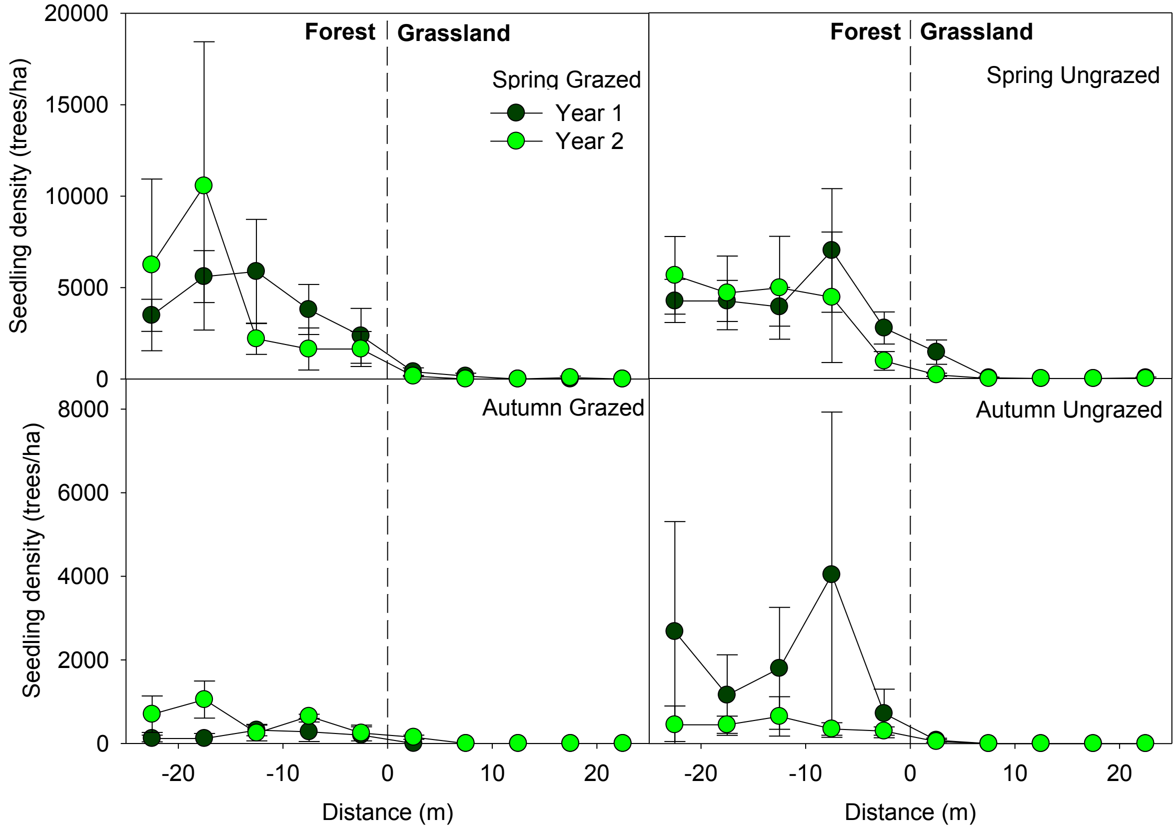

Figure 5.2. Mean seedling density (with standard error) by year for each season x management combination (spring grazed and ungrazed, and autumn grazed and ungrazed). In each graph, forest areas are -25-0, and grasslands are 0-25 m (forest-grassland edge is denoted by dashed line). Note different scales of seedling density for spring and autumn.

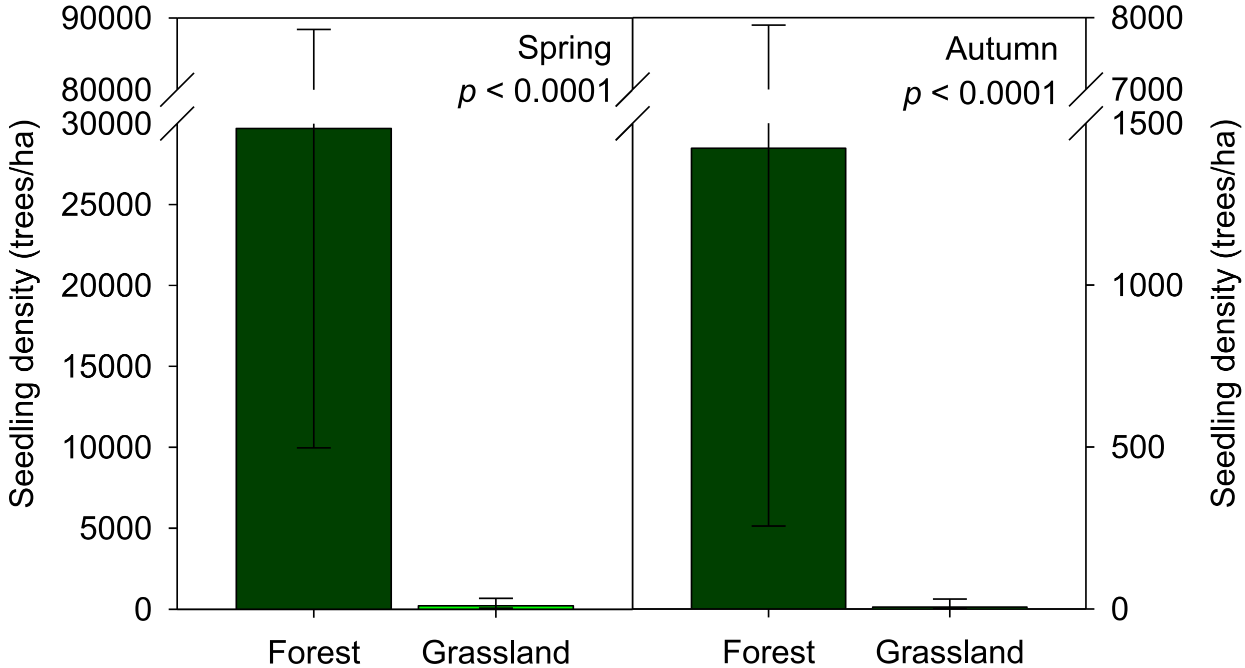

Figure 5.3. Mean seedling density (with 95% confidence intervals) by habitat. Note that analyses were done separately by season (as denoted by *p*-values), and scales differ with season.

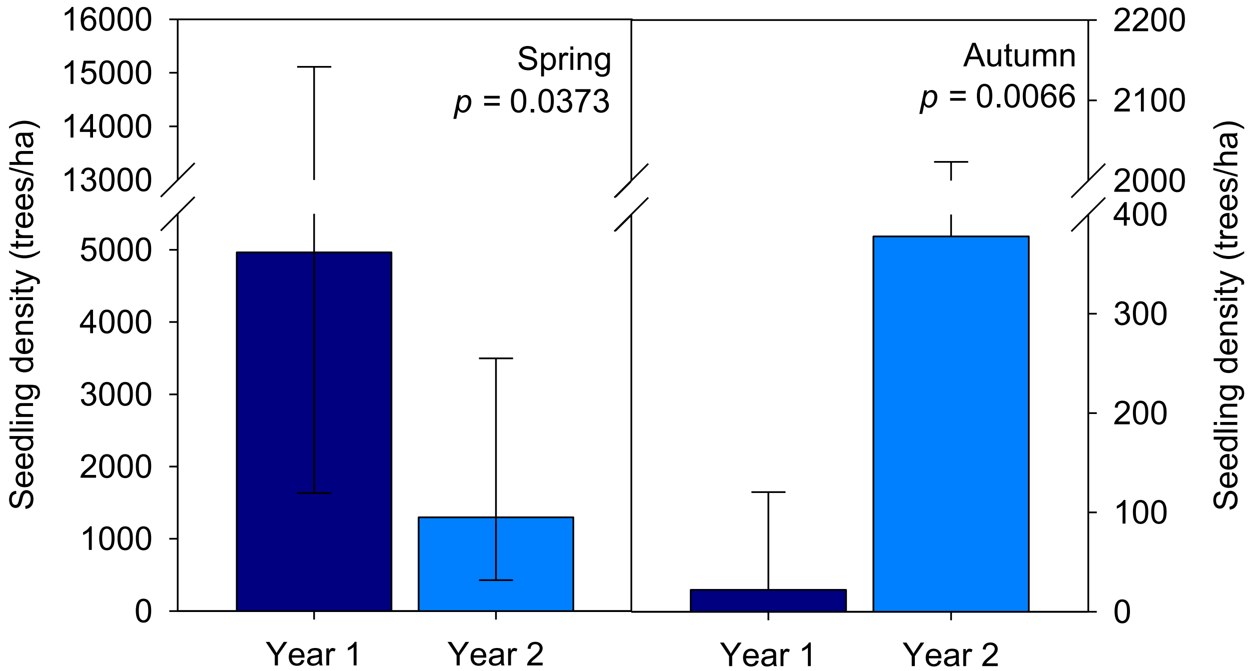

Figure 5.4. Mean seedling density (with 95% confidence intervals) by year. Note that analyses were done separately by season (as denoted by *p*-values).

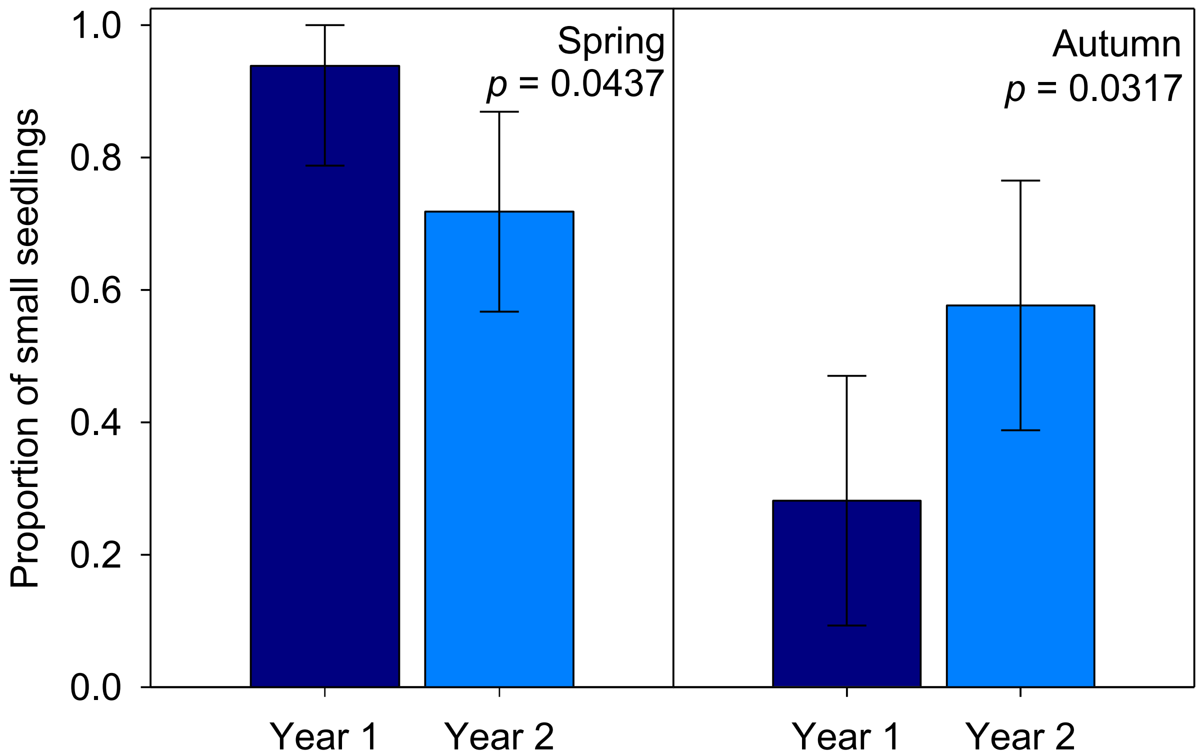

Figure 5.5. Proportion of small (< 10 cm tall) seedlings in spring (left) and autumn (right) by sample year. Bars are means with 95% confidence intervals.

Chapter 6
Synthesis of agroforestry impacts to vegetation and soils dynamics

Comparing vegetation richness and composition between agroforestry management phases, I found the greatest differences between Grasslands and Mid-stage forests, likely because the low light availability and thick litter layer associated with Mid-stage forests prevented some plant species from surviving in this relatively new environment (Chapter 2). This stage in *Eucalyptus grandis* forest coincided with the development stage of *Pinus taeda* in Chapter 4 examining the individual and interactive effects of afforestation and grazing on understory vegetation richness. I found that species richness was higher in grasslands than forests at small-scales, but because of the patchy distribution of vegetation in forests, species richness was similar at larger scales. And the effects of grazing and habitat were interactive: the removal of grazing in grasslands decreased species richness, but increased richness in forests. This finding is similar to previous research supporting the intermediate disturbance hypothesis: when grazing is removed, competitive species become dominant and total richness is reduced (Grime 1973, Connell 1978, Fox 1979, Altesor *et al.* 2005, Altesor *et al.* 2006). In forests, because understory vegetation tends to be relatively sparse (Rummel 1951, Cooper 1960, Fleischner 1994), exclusion of species by dominant competitors is less likely to occur; instead, the elimination of grazing in the forests likely enabled species sensitive to grazing pressure to increase. Additional vegetation change may occur with multiple forest rotations, however, as multiple short rotations may cause a decrease in species richness and diversity (Wen *et al.* 2010).

While vegetation change is often related to changes in soils characteristics (Kimmins 1997, Paul *et al.* 2002, Browning *et al.* 2008), I did not find changes in soils characteristics that were consistent with agroforestry management (Chapter 3), and therefore, no clear relation between soils characteristics and changes in vegetation dynamics. This may be due to limitation in sample size, and I anticipate significant changes in soils dynamics if soils were more intensely sampled, both spatially and temporally, especially for soil moisture (Fu *et al.* 2000, Farley *et al.* 2005), bulk density and organic matter (Shirato *et al.* 2004, del Pilar Clavijo *et al.* 2005, Korkanc *et al.* 2009), and nitrogen (Parfitt *et al.* 1997, Saviozzi *et al.* 2001). Some research in afforested systems has suggested that soil properties through the first rotation are similar to conditions during the previous land use (Binkley *et al.* 2004), and it may take more than one rotation of forest for changes in soil nutrition to be evident; the full impact of afforestation on soil quality, therefore, may not be understood immediately (Jackson *et al.* 2000).

Conifer germination and establishment occurred within plantations, but seedlings were rare in grasslands (Chapter 5). While *Pinus taeda* is exotic to Uruguay and to the larger Campos ecoregion, it does not seem to pose an immediate threat of encroachment into remnant grasslands within the forested landscape. While disturbances can result in invasions of exotic species, the Campos has a low proportion of exotic species overall, and the addition of exotic tree species, surprisingly, does not seem to offer any advantage to exotic species (Hobbs & Huenneke 1992). While I did not find an immediate threat of conifer encroachment into remnant grasslands, as plantations remain on the landscape in the long-term, shade from trees and sheltering from extreme temperatures may facilitate future seedling establishment in plantation-adjacent grasslands (Fulco *et al.* 2001, Siemann & Rogers 2003, Coop & Givnish 2008).

The configuration of forest plantations and the land use history in the Campos may make vegetation more resilient to effects of afforestation than in other ecosystems. Given that plants have already adapted to long-term grazing (Greenberg *et al.* 1995, Li *et al.* 2007), many adaptations have developed, such as wind-dispersed seeds and versatile rosette morphology, that serve plants well in afforested systems, where wind dispersed seeds can colonize open spaces, and the versatile architecture of rosettes (flat structure when not flowering) can take better advantage of resources, like reduced light (McIntyre *et al.* 1995). Also, the inclusion of fairly large areas of grassland within the plantation forest landscape provides a consistent reservoir of grassland plants that can colonize plantation areas after tree harvest. Generally half the land within afforested areas remains in grazed grassland (J. Posse, Weyerhaeuser Uruguay, pers. comm.). Therefore , vegetation may have a greater chance of recovery following an afforestation cycle (Baum *et al.* 2009).

The interaction between land-use history, soils and vegetation characteristics is known to shape vegetation community dynamics (Cramer *et al.* 2008). While my research does not demonstrate a strong link with soils characteristics, land-use history and the recent advent of afforestation in this region certainly interact with vegetation characteristics to determine vegetation dynamics. With the combination of chronic grazing and recent afforestation, I demonstrate that vegetation richness and composition are at least temporarily altered, but some recovery occurs following plantation thinning and harvest. However, the full extent of the effect of afforestation on grassland vegetation remains unclear. The Campos represents an ecosystem that has been shaped by long-term human activities; grazing. The addition of agroforestry to this system will likely result in a hybridization of historical and new characteristics (Hobbs *et al.* 2009).

REFERENCES

Adams, M. L., M. R. Davis, and K. J. Powell. 2001. Effects of grassland afforestation on exchangeable soil and soil solution aluminum. Australian Journal of Soil Research **39**:1003-1014.

Alrababah, M. A., M. A. Alhamad, A. Suwaileh, and M. Al-Gharaibeh. 2007. Biodiversity of semi-arid Mediterranean grasslands: Impact of grazing and afforestation. Applied Vegetation Science **10**:257-264.

Altesor, A., E. Di Landro, H. May, and E. Ezcurra. 1998. Long-term species change in a Uruguayan grassland. Journal of Vegetation Science **9**:173-180.

Altesor, A., M. Oesterheld, E. Leoni, F. Lezama, and C. Rodriguez. 2005. Effect of grazing on community structure and productivity of Uruguayan grassland. Plant Ecology **179**:83-91.

Altesor, A., F. Pezzani, S. Grun, and C. Rodriguez. 1999. Relationship betwen strategies and morphological attributes in an Uruguayan grassland: a functional approach. Journal of Vegetation Science **10**:457-462.

Altesor, A., G. Pineiro, F. Lezama, R. B. Jackson, M. Sarasola, and J. M. Paruelo. 2006. Ecosystem changes associated with grazing in subhumid South American grasslands. Journal of Vegetation Science **17**:323-332.

Attiwill, P. M. 1994a. Ecological disturbance and the conservative management of Eucalypt forests in Australia. Forest Ecology and Management **63**:301-346.

Attiwill, P. M. 1994b. The disturbance of forest ecosystems - the ecological basis for conservative management. Forest Ecology and Management **63**:247-300.

Austrheim, G., A. Mysterud, B. Pedersen, R. Halvorsen, K. Hassel, and M. Evju. 2008. Large scale experimental effects of three levels of sheep densities on an alpine ecosystem. Oikos **117**:837-846.

Bakker, J. D., F. Rudebusch, and M. M. Moore. 2010. Effects of long-term livestock grazing and habitat on understory vegetation. Western North American Naturalist **70**:334-344.

Baldi, G., J. P. Guerschman, and J. M. Paruelo. 2006. Characterizing fragmentation in temperate South America grasslands. Agriculture, Ecosystems and Environment **116**:197-208.

Baldi, G., and J. M. Paruelo. 2008. Land-use and land cover dynamics in South American temperate grasslands. Ecology and Society **13**:article number 6.

Baum, S., M. Weih, G. Busch, F. Kroiher, and A. Bolte. 2009. The impact of short rotation coppice plantations in phytodiversity. Landbauforschung Volkenrode **59**:163-170.

Beever, E. A., R. J. Tausch, and W. E. Thogmartin. 2008. Multi-scale responses of vegetation to removal of horse grazing from Great Basin (USA) mountain ranges. Plant Ecology **196**:163-184.

Berretta E.J. Country Pasture/Forage Resources Profiles: Uruguay, Agriculture Sector. 2003. FAO (Food and Agriculture Organization of the United Nations). 5-2-2012.

Berretta, E. J., D. F. Risso, F. Montossi, and G. Pigurina. 2000. Campos in Uruguay. Pages 377-394 *in* G. Lemaire, J. Hodgson, A. de Moraes, C. Nabinger, and P. C. d. F. Carvalho editors. Grassland Ecophysiology and Grazing Ecology.

Berthrong, S. T., C. W. Schadt, G. Pineiro, and R. B. Jackson. 2009. Afforestation Alters the Composition of Functional Genes in Soil and Biogeochemical Processes in South American Grasslands. Applied and Environmental Microbiology **75**:6240-6248.

Binkley, D., J. Kaye, M. Barry, and M. G. Ryan. 2004. First-rotation changes in soil carbon and nitrogen in a Eucalyptus plantation in Hawaii. Soil Science Society of America Journal **68**:1713-1719.

Bokdam, J., and J. M. Gleichman. 2000. Effects of grazing by free-ranging cattle on vegetation dynamics in a continental north-west European heathland. Journal of Applied Ecology **37**:415-431.

Booth, M. S., J. M. Stark, and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological Monographs **75**:139-157.

Boulant, N., A. Garnier, T. Curt, and J. Lepart. 2009. Disentangling the effects of land use, shrub cover and climate on the invasion speed of native and introduced pines in grasslands. Diversity and Distributions **15**:1047-1059.

Brady N. C., and R. R. Weil. 2008. The Nature and Properties of Soils., 14 edition. Pearson Prentice Hall, Upper Saddle River.

Bremer, L. L., and K. A. Farley. 2010. Does plantation forestry restore biodiversity or create green deserts? A synthesis of the effects of land-use transitions on plant species richness. Biodiversity and Conservation **19**:3893-3915.

Briske, D. D., S. D. Fuhlendorf, and F. E. Smeins. 2003. Vegetation dynamics on rangelands: a critique of the current paradigms. Journal of Applied Ecology **40**:601-614.

Brockerhoff, E. G., H. Jactel, J. A. Parrotta, C. P. Quine, and J. Sayer. 2008. Plantation forests and biodiversity: oxymoron or opportunity? Biodiversity and Conservation **17**:925-951.

Brown, S., and A. E. Lugo. 1990. Effects of forest clearing and succession on the carbon and nitrogen content of soils in Puerto Rico and US Virgin Islands. Plant and Soil **124**:53-64.

Browning, D. M., S. R. Archer, G. P. Asner, M. P. McClaran, and C. A. Wessman. 2008. Woody plants in grasslands: post-encroachment stand dynamics. Ecological Applications **18**:928-944.

Brudvig, L. A., and E. I. Damschen. 2011. Land-use history, historical connectivity, and land management interact to determine longleaf pine woodland understory richness and composition. Ecography **34**:257-266.

Buckley, Y. M., E. Brockerhoff, L. Langer, N. Ledgard, H. North, and M. Rees. 2005. Slowing down a pine invasion despite uncertainty in demography and dispersal. Journal of Applied Ecology **42**:1020-1030.

Buscardo, E., G. F. Smith, D. L. Kelly, H. Freitas, S. Iremonger, F. J. G. Mitchell, S. O'Donoghue, and A. M. Mckee. 2008. The early effects of afforestation on biodiversity of grasslands in Ireland. Biodiversity and Conservation **17**:1057-1072.

Carrere, R., and L. Lohmann. 1996. Uruguay: 'forests' on the grasslands. Pages 186-197 *in* Pulping the south: industrial tree plantations and the world paper economy. Zed Books Ltd, London.

Chaneton, E. J., and J. M. Facelli. 1991. Effects of plant community diversity: spatial scales and dominance hierarchies. Vegetatio **93**:143-155.

Chapin, F. I. S., G. R. Shaver, A. E. Giblin, K. J. Nadelhoffer, and J. A. Laundre. 1995. Responses of arctic tundra to experimental and observed changes in climate. Ecology **76**:694-711.

Chen, C. R., L. M. Condron, M. R. Davis, and R. R. Sherlock. 2000. Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. Plant and Soil **220**:151-163.

Chescheir, G. M., R. W. Skaggs, and D. M. Amatya. 2008. Hydrologic impacts of converting grassland to managed forest in Uruguay. Pages 1-9 *in*.

Cingolani, A. M., M. R. Cabido, D. Renison, and V. N. Solis. 2003. Combined effects of environment and grazing on vegetation structure in Argentine granite grasslands. Journal of Vegetation Science **14**:223-232.

Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science **280**:745-747.

Colwell, R. K., and J. A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London Series B - Biological Sciences **345**:101-118.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science **199**:1302-1310.

Coop, J. D., and T. J. Givnish. 2008. Constraints on tree seedling establishment in montane grasslands of the Valles Caldera, New Mexico. Ecology **89**:1101-1111.

Cooper, C. F. 1960. Changes in vegetation, structure, and growth of southwestern pine forests since white settlement. Ecological Monographs **30**:129-164.

Copenheaver, C. A., N. E. Fuhrman, L. S. Gellerstedt, and P. A. Gellerstedt. 2004. Tree encroachment in forest openings: a case study from Buffalo Mountain, Virginia. Castanea **69**:297-308.

Cornell, H. V., and J. H. Lawton. 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. Journal of Animal Ecology **61**:1-12.

Coughenour, M. B. 1991. Invited synthesis paper: spatial components of plant-herbivore interactions in pastoral, ranching, and native ungulate ecosystems. Journal of Range Management **44**:530-542.

Cramer, V. A., R. J. Hobbs, and R. J. Standish. 2008. What's new about old fields? Land abandonment and ecosystem assembly. Trends in Ecology & Evolution **23**:104-112.

Crawley, M. J., and J. E. Harral. 2001. Scale dependence in plant biodiversity. Science **291**:864-868.

Cuevas, Y. A., and S. M. Zalba. 2010. Recovery of native grasslands after removing invasive pines. Restoration Ecology **18**:711-719.

Darwin C. 2009. The voyage of the beagle., Anniversary edition. National Geographic, Washington DC.

Davis, M. R., and L. M. Condron. 2002. Impact of grassland afforestation on soil carbon in New Zealand: a review of paired-site studies. Australian Journal of Soil Research **40**:675-690.

del Pilar Clavijo, M., M. Nordenstahl, P. E. Gundel, and E. G. Jobbagy. 2005. Poplar afforestation effects on grassland structure and composition in the flooding pampas. Rangeland Ecology and Management **58**:474-479.

Denslow, J. S. 1995. Disturbance and diversity in tropical rain forests - the density effect. Ecological Applications **5**:962-968.

Dickie, I. A., G. W. Yeates, M. G. St.John, B. A. Stevenson, J. T. Scott, M. C. Rillig, D. A. Peltzer, K. H. Orwin, M. U. F. Kirschbaum, J. E. Hunt, L. E. Burrows, M. M. Barbour, and J. Aislabie. 2011. Ecosystem service and biodiversity trade-offs in two woody successions. Journal of Applied Ecology **48**:926-934.

Dorrough, J. W., J. E. Ash, S. Bruce, and S. McIntyre. 2007. From plant neighborhood to landscape scales: how grazing modifies native and exotic plant species richness in grassland. Plant Ecology **191**:185-198.

Dovciak, M., R. Hrivnak, K. Ujhazy, and D. Gomory. 2008. Seed rain and environmental controls on invasion of Picea abies into grassland. Plant Ecology **194**:135-148.

Drake, P. L., D. S. Mendham, D. S. White, and G. N. Ogden. 2009. A comparison of growth, photosynthetic capacity and water stress in Eucalyptus globulus coppice regrowth and seedlings during early developement. Tree Physiology **29**:663-674.

Duarte, L. d. S., R. E. Machado, S. M. Hartz, and V. D. Pillar. 2006. What saplings can tell us about forest expansion over natural grasslands. Journal of Vegetation Science **17**:799-808.

Dumig, A., P. Schad, C. Rumpel, M.-F. Dignac, and I. Kogel-Knabner. 2008. *Araucaria* forest expansion on grassland in the southern Brazilian highlands as revealed by 14C and carbon isotope 13 studies. Geoderma **145**:143-157.

Dunwiddie, P. W. 1977. Recent tree invasion of subalpine meadows in the Wind River Mountains, Wyoming. Arctic and Alpine Research **9**:393-399.

Dupouey, J. L., E. Dambrine, J. D. Laffite, and C. Moares. 2002. Irreversible impact of past land use on forest soils and biodiversity. Ecology **83**:2978-2984.

Dzwonko, Z., and S. Loster. 1997. Effects of dominant trees and anthropogenic disturbances on species richness and floristic composition of secondary communities in southern Poland. Journal of Applied Ecology **34**:861-870.

EFI. 2000. NEWFOR - New forests for Europe: Afforestation at the turn of the century., EFI Proceedings No 35 edition. European Forest Institute, Freiburg, Germany.

Evju, M., G. Austrheim, R. Halvorsen, and A. Mysterud. 2009. Grazing responses in herbs in relation to herbivore selectivity and plant traits in an alpine ecosystem. Oecologia **161**:77-85.

Evrendilek, F., I. Celik, and S. Kilic. 2004. Changes in soil organic carbon and other physical soil properties along adjacent Mediterranean forest, grassland, and cropland ecosystems in Turkey. Journal of Arid Environments **59**:743-752.

Eycott, A. E., A. R. Watkinson, and P. M. Dolman. 2006. Ecological patterns of plant diversity in a plantation forest managed by clearfelling. Journal of Applied Ecology **46**:1160-1171.

Facelli, J. M., and S. T. A. Pickett. 1991. Plant litter - its dynamics and effects on plant community structure. Botanical Review **57**:1-32.

Fahrig, L., D. P. Coffin, W. K. Lauenroth, and H. H. Shugart. 1994. The advantage of long-distance clonal spreading in highly disturbed habitats. Evolutionary Ecology **8**:172-187.

FAO. 2010. Global Forest Resources Assessment 2010., 163 edition. Food and Agriculture Organization of the United Nations, Rome.

Farley, K. A., E. G. Jobbagy, and R. B. Jackson. 2005. Effects of afforestation on water yield: a global synthesis with implications for policy. Global Change Biology **11**:1565-1576.

Fernandez-Gimenez, M., and B. len-Diaz. 2001. Vegetation change along gradients from water sources in three grazed Mongolian ecosystems. Plant Ecology **157**:101-118.

Fidelis, A., C. C. Blanco, S. C. Muller, Pillar Valerio D., and J. Pfadenhauer. 2012. Short-term changes caused by fire and mowing in Brazilian Campos grasslands with different long-term fire histories. Journal of Vegetation Science **23**:552-562.

Fleischner, T. L. 1994. Ecological costs of livestock grazing in western North America. Conservation Biology **8**:629-644.

Foth H. D., and J. W. Schafer. 1980. Soil geography and land use. John Wiley & Sons, New York.

Fox, J. F. 1979. Intermediate-disturbance hypothesis. Science **204**:1344-1345.

Franzluebbers, A. J., J. A. Stuedemann, H. H. Schomberg, and S. R. Wilkinson. 2000. Soil organic C and N pools under long-term pasture management in the Southern Piedmont USA. Soil Biology and Biogeochemistry **32**:469-478.

Fu, B. J., L. D. Chen, K. M. Ma, H. F. Zhou, and J. Wang. 2000. The relationships between land use and soil conditions in the hilly area of the loess plateau in northern Shaanxi, China. Catena **39**:69-78.

Fuhlendorf, S. D., and F. E. Smeins. 1996. Spatial scale influence on longterm temporal patterns of a semi-arid grassland. Landscape Ecology **11**:107-113.

Fuhlendorf, S. D., and F. E. Smeins. 1999. Scaling effects of grazing in a semi-arid grassland. Journal of Vegetation Science **10**:731-738.

Fulco, L., H. de Kroon, H. H. T. Prins, and F. Berendse. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. Journal of Vegetation Science **12**:579-588.

Garcia-Oliva, F., I. Casar, P. Morales, and J. M. Maass. 1994. Forest-to-pasture conversion influences on soil organic carbon dynamics in a tropical deciduous forest. Oecologia **99**:392-396.

Gautreau, P. 2010. Rethinking the dynamics of woody vegetation in Uruguayan campos, 1800-2000. Journal of Historical Geography **36**:194-204.

Geary, T. F. 2001. Afforestation in Uruguay: study of a changing lanscape. Journal of Forestry **99**:35-39.

Gibson D. J. 2009. Grasses and Grassland Ecology. Oxford University Press, Oxford.

Giddens, K. M., R. L. Parfitt, and H. J. Percival. 1997. Comparison of some soil properties under Pinus radiata and improved pasture. New Zealand Journal of Agricultural Research **40**:409-416.

Greenberg, C. H., D. G. Neary, L. D. Harris, and S. P. Linda. 1995. Vegetation recovery following high-intensity wildfire and silvicultural treatment in sand pine scrub. American Midland Naturalist **133**:149-163.

Grime, J. P. 1973. Control of species density in herbaceous vegetation. Journal of Environmental Management **1**:151-167.

Guerschman, J. P., and J. M. Paruelo. 2005. Agricultural impacts on ecosystem functioning in temperate areas of North and South America. Global Planetary Change **47**:170-180.

Guo, L. B., and R. M. Gifford. 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology **8**:345-360.

Guo, Q. F. 2004. Slow recovery in desert perennial vegetation following prolonged human disturbance. Journal of Vegetation Science **15**:757-762.

Hartnett, D. C., K. R. Hickman, and L. E. F. Walter. 1996. Effects of bison grazing, fire, and topography on floristic diversity in tallgrass prairie. Journal of Range Management **49**:413-420.

He, Z., W. Zhao, X. Chang, X. Chang, and J. Fang. 2006. Scale dependence in desert plant diversity. Biodiversity and Conservation **15**:3055-3064.

Hobbs, R. J., S. Arico, J. Aronson, J. A. Baron, P. Bridgewater, V. A. Cramer, P. R. Epstein, J. J. Ewel, C. A. Klink, A. E. Lugo, D. Norton, D. Ojima, D. M. Richardson, E. W. Sanderson, F. Valladares, M. Vila,

R. Zamora, and M. Zobel. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography **15**:1-7.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution **24**:599-605.

Hobbs, R. J., and L. F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology **6**:324-337.

House, J. I., S. Archer, D. D. Breshears, R. J. Scholes, and NCEAS Tree-Grass Interactions Participants. 2003. Conundrums in mixed woody-herbaceous plant systems. Journal of Biogeography **30**:1763-1777.

Huang, Z. Q., M. R. Davis, L. M. Condron, and P. W. Clinton. 2011. Soil carbon pools, plant biomarkers, and mean carbon residence time after afforestation of grassland with three tree species. Soil Biology & Biochemistry **43**:1341-1349.

Hughes, S., D. A. Norris, P. A. Stevens, B. Reynolds, T. G. Williams, and C. Woods. 1994. Effects of forest age on surface drainage water and soil solution aluminum chemistry in stagnopodzols in Wales. Water, Air, and Soil Pollution **77**:115-139.

INIA. Agroclimatic bank of INIA stations. 2011. 11-25-2011.

Ito, S., R. Nakayama, and G. P. Buckley. 2004. Effects of previous land-use on plant species diversity in semi-natural and plantation forests in a warm-temperate region in southeastern Kyushu, Japan. Forest Ecology and Management **196**:213-225.

Izhaki, I., N. Henig-Sever, and G. Ne'erman. 2000. Soil seed banks in Mediterranean Aleppo pine forests: the effect of heat, cover, and ash on seedling emergence. Journal of Ecology **88**:667-675.

Jackson, R. B., J. Canadell, J. R. Ehleringer, H. A. Mooney, O. E. Sala, and E. D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia **108**:389-411.

Jackson, R. B., H. J. Schenk, E. G. Jobbagy, J. Canadell, G. D. Colello, R. E. Dickinson, C. B. Field, P. Friedlingstein, M. Heimann, K. Hibbard, D. W. Kicklighter, A. Kleidon, R. B. Nielson, W. J. Parton, O. E. Sala, and M. T. Sykes. 2000. Belowground consequences of vegetation change and their treatment in models. Ecological Applications **10**:470-483.

Johnson, S. R., and D. R. Young. 1993. Factors contributing to the decline of Pinus taeda on a Viriginia barrier island. Bulletin of the Torrey Botanical Club **120**:431-438.

Johnson, W. M. 1956. The effect of grazing intensity on plant composition, vigor, and growth of pine-bunchgrass ranges in central Colorado. Ecology **37**:790-798.

Kallimanis, A. S., J. M. Halley, D. Vokou, and S. P. Sgardelis. 2008. The scale of analysis determines the spatial pattern of woody species diversity in the Mediterranean environment. Plant Ecology **196**:143-151.

Keeley, J. E., D. Lubin, and C. J. Fotheringham. 2003. Fire and grazing impacts on plant diversity and alien plant invasions in the southern Sierra Nevada. Ecological Applications **13**:1355-1374.

Kimmins, J. P. 1997. The least renewable physical component of the ecosystem. Pages 228-268 *in* Forest ecology: a foundation for sustainable management. Prentice Hall, Upper Saddle River, New Jrrsey.

Kisselle, K. W., R. G. Zepp, R. A. Burke, A. D. Pinto, M. M. C. Bustamante, S. Opsahl, R. F. Varella, and L. T. Viana. 2002. Seasonal soil fluxes of carbon monoxide in burned and unburned Brazilian savannas. Journal of Geophysical Research **107**:8051.

Knapp, A. K., P. A. Fay, J. M. Blair, S. L. Collins, M. D. Smith, J. D. Carlisle, C. W. Harper, B. T. Danner, M. S. Lett, and J. K. McCarron. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science **298**:2202-2205.

Kohler, F., F. Gillet, J. M. Gobat, and A. Buttler. 2004. Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. Journal of Vegetation Science **15**:143-150.

Korkanc, S. Y., A. Hizal, and N. Ozyuvaci. 2009. Effects of land-use change on some hydro-physical properties of soils. Fresenius Environmental Bulletin **18**:1730-1735.

Laclau, J. P., P. Deleporte, J. Ranger, J. P. Bouilleti, and G. Kazotti. 2003. Nutrient dynamics throughout the rotation of Eucalyptus clonal stands in Congo. Annals of Botany **91**:879-892.

Laclau, J. P., J. Ranger, P. Deleporte, Y. Nouvellon, L. Saint-Andre, S. Marlet, and J. P. Bouillet. 2005. Nutrient cycling in a clonal stand of Eucalyptus and an adjacent savanna ecosystem in Congo 3. Input-output budgets and consequences for the sustainability of the plantations. Forest Ecology and Management **210**:375-391.

Lande, R., P. J. DeVries, and T. R. Walla. 2000. When species accumulation curves intersect: implications for ranking diversity using small samples. Oikos **89**:601-605.

Landsberg, J., C. D. James, J. Maconochie, A. O. Nicholls, J. Stol, and R. Tynan. 2002. Scale-related effects of grazing on native plant communities in an arid rangeland region of South Australia. Journal of Applied Ecology **39**:427-444.

Le Bagousse-Pinguet, Y., E. M. Gross, and D. Straile. 2012. Release from competition and protection determine the outcome of plant interactions along a grazing gradient. Oikos **121**:95-101.

Ledgard, N. 2001. The spread of lodgepole pine (Pinus contorta, Dougl.) in New Zealand. Forest Ecology and Management **141**:43-57.

Li, J., J. A. Duggin, W. A. Loneragan, and C. D. Grant. 2007a. Grassland responses to multiple disturbances on the New England Tablelands in NSW, Australia. Plant Ecology **193**:39-57.

Lindenmayer, D. B., J. Fischer, A. Felton, M. Crane, D. Michael, C. Macgregor, R. Montague-Drake, A. Manning, and R. J. Hobbs. 2008. Novel ecosystems resulting from landscape transformation can create dilemmas for modern conservation practice. Conservation Letters **1**:129-135.

Littell R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models., Second Edition edition. SAS Institute Inc., Cary, North Carolina.

Loumeto, J. J., and C. Huttel. 1997. Understory vegetation in fast-growing tree plantations on savanna soils in Congo. Forest Ecology and Management **99**:65-81.

Marini, L., P. Fontana, A. Battisti, and K. J. Gaston. 2009. Agricultural management, vegetation traits and landscape drive orthopteran and butterfly diversity in a grassland-forest mosaic: a multi-scale approach. Insect Conservation and Diversity **2**:213-220.

Marques, M. C. M., and P. E. A. M. Oliveira. 2008. Seasonal rhythms of seed rain and seedling emergence in two tropical rain forests in southern Brazil. Plant Biology **10**:596-603.

Mather, A. 1993. Introduction. Pages 1-12 *in* A. Mather editor. Afforestation: policies, planning, and progress. Belhaven Press, London.

McCune B., and J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach.

McIntyre, S., S. Lavorel, and R. M. Tremont. 1995. Plant life-history attributes - their relationship to disturbance responses in herbaceous vegetation. Journal of Ecology **83**:31-44.

Mendham, D. S., A. M. O'Connell, T. S. Grove, and S. J. Rance. 2003. Residue management effects on soil carbon and nutrient contents and growth of second rotation Eucalypts. Forest Ecology and Management **181**:357-372.

Merino, A., A. Rodriguez Lopez, J. Branas, and R. Rodriguez-Soalleiro. 2003. Nutrition and growth in newly established plantations of Eucalyptus globulus in northwestern Spain. Annals of Forest Science **60**:509-517.

Milchunas, D. G., O. E. Sala, and W. K. Lauenroth. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. The American Naturalist **132**:87-106.

Milchunas, D. G., and W. K. Lauenroth. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. Ecological Monographs **63**:327-366.

Munhoz, C. B. R., J. M. Felfili, and C. Rodrigues. 2008. Species-environment relationship in the herb-subshrub layer of a moist Savanna site, Federal District, Brazil. Brazilian Journal of Biology **68**:25-35.

Naumburg, E., and L. E. DeWald. 1999. Relationships between Pinus ponderosa forest structure, light characteristics, and understory graminoid species presence and abundance. Forest Ecology and Management **124**:205-215.

Neff, J. C., R. L. Reynolds, J. Belnap, and P. Lamothe. 2005. Multi-decadal impacts of grazing on soil physical and biogeochemical properties in southeast Utah. Ecological Applications **15**:87-95.

Nosetto, M. D., E. G. Jobbagy, and J. M. Paruelo. 2005. Land-use change and water losses: the case of grassland afforestation across a soil textural gradient in central Argentina. Global Change Biology **11**:1101-1117.

Odion, D. C., and D. A. Sarr. 2007. Managing disturbance regimes to maintain biological diversity in forested ecosystems of the Pacific Northwest. Forest Ecology and Management **246**:57-65.

Olff, H., and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. Tree **13**:261-265.

Olmos, V. M., and J. P. Siry. 2009. Economic impact evaluation of Uruguay forest sector development policy. Journal of Forestry **107**:63-68.

Onaindia, M., I. Dominguez, I. Albizu, C. Garbisu, and I. Amezaga. 2004. Vegetation diversity and vertical structure as indicators of forest disturbance. Forest Ecology and Management **195**:341-354.

Oosterhoorn, M., and M. Kappelle. 2000. Vegetation structure and composition along an interior-edge-exterior gradient in a Costa Rican montane cloud forest. Forest Ecology and Management **126**:291-307.

Overbeck, G. E., S. C. Muller, A. Fidelis, J. Pfadenhauer, V. D. Pillar, C. C. Blanco, I. I. Boldrini, R. Both, and E. D. Forneck. 2007. Brazil's neglected biome: the South American Campos. Perspectives in Plant Ecology, Evolution, and Systematics **9**:101-116.

Pallares O.R., Berretta E.J. & Maraschin G.E. The South American Campos ecosystem. Suttie, J. M., Reynolds, S. G., and Batello, C. Grasslands of the World. 2005. Plant Production and Protection Series No. 34.

Palmer, M. W., and P. S. White. 1994. Scale dependence and the species-area relationship. The American Naturalist **144**:717-740.

Papanastasis, V., Z. Koukoura, D. Alifragis, and I. Makedos. 1995. Effects of thinning, fertilization, and sheep grazing on the understory vegetation of *Pinus pinaster* plantations. Forest Ecology and Management **77**:181-189.

Parada, T., and C. H. Lusk. 2011. Patterns of tree seedling mortality in a temperate-mediterranean transition zone forest in Chile. Gayana Botanica **68**:236-243.

Parfitt, R. L., H. J. Percival, R. A. Dahlgren, and L. F. Hill. 1997. Soil and soil solution chemistry under pasture and radiata pine in New Zealand. Plant and Soil **191**:279-290.

Parrotta, J. A. 1999. Productivity, nutrient cycling, and succession in single- and mixed-species plantations of Casuarina equisetifolia, Eucalyptus robusta, and Leucaena leucocephala in Puerto Rico. Forest Ecology and Management **124**:45-77.

Parsons M., M. Gavran, and J. Davidson. 2006. Australia's plantations. Department of Agriculture, Fisheries and Forestry, Canberra.

Paruelo, J. M., E. G. Jobbagy, M. Oesterheld, R. A. Golluscio, and M. R. Aguiar. 2007. The grasslands and steppes of Patagonia and the Rio de la Plata plains. Pages 232-248 *in* T. T. Veblen, K. R. Young, and A. R. Orme editors. The physical geography of South America. Oxford University Press, New York.

Paul, K. I., P. J. Polglase, J. G. Nyakuengama, and P. K. Khanna. 2002. Change in soil carbon following afforestation. Forest Ecology and Management **168**:241-257.

Pickett, S. T. A. 1989. Space-for-time substitution as an alternative to long-term studies. Pages 110-135 *in* G. E. Likens editor. Long-term studies in ecology: approaches and alternatives. Springer-Verlag, New York.

Pollock, M. L., C. J. Legg, J. P. Holland, and C. M. Theobald. 2007. Assessment of expert opinion: seasonal sheep preference and plant response to grazing. Rangeland Ecology & Management **60**:125-135.

Powers, J. S., J. P. Haggar, and R. F. Fisher. 1997. The effect of overstory composition on understory woody regeneration and species richness in 7-year-old plantations in Costa Rica. Forest Ecology and Management **99**:43-54.

Reitalu, T., L. J. Johansson, M. T. Sykes, K. Hall, and H. C. Prentic. 2010. History matters: village distances, grazing, and grassland species diversity. Journal of Applied Ecology **47**:1216-1224.

Richardson, D. M., and W. J. Bond. 1991. Determinants of plant distribution: evidence from pine invasions. The American Naturalist **137**:639-668.

Romermann, C., M. Bernhardt-Romermann, M. Kleyer, and P. Poschlod. 2009. Substitutes for grazing in semi-natural grasslands - do mowing or mulching represent valuable alternatives to maintain vegetation structure? Journal of Vegetation Science **20**:1086-1098.

Ross, K. A., J. E. Taylor, M. D. Fox, and B. J. Fox. 2004. Interaction of multiple disturbances: importance of disturbance interval in the effects of fire on rehabilitating mined areas. Austral Ecology **29**:508-529.

Rummel, R. S. 1951. Some effects of livestock grazing on ponderosa pine forest and range in central Washington. Ecology **32**:594-607.

Savadogo, P., M. Tigabu, L. Sawadogo, and P. C. Oden. 2009. Examination of multiple disturbances effects on herbaceous vegetation communities in the Sudanian savanna-woodland of West Africa. Flora **204**:409-422.

Saviozzi, A., R. Levi-Minzi, R. Cardelli, and R. Riffaldi. 2001. A comparison of soil quality in adjacent cultivated, forest, and native grassland soils. Plant and Soil **233**:251-259.

Schenk, H. J., and R. B. Jackson. 2002. The global biogeography of roots. Ecological Monographs **72**:311-328.

Schulze, E. D., H. A. Mooney, O. E. Sala, E. Jobbagy, N. Buchmann, G. Bauer, J. Canadell, R. B. Jackson, J. Loreti, M. Oesterheld, and J. R. Ehleringer. 1996. Rooting depth, water availability, and vegetation cover along an aridity gradient in Patagonia. Oecologia **108**:503-511.

Scimone, M., A. J. Rook, J. P. Garel, and N. Sahin. 2007. Effects of livestock breed and grazing intensity on grazing systems: 3. Effects on diversity of vegetation. Grass and Forage Science **62**:172-184.

Scott, D. F., and W. Lesch. 1997. Streamflow responses to afforestation with Eucalyptus grandis and Pinus patula and to felling in the Mokobulaan experimental catchments, South Africa. Journal of Hydrology **199**:360-377.

Seastedt, T. R., R. J. Hobbs, and K. N. Suding. 2008. Management of novel ecosystems: are novel approaches required? Frontiers in Ecology and the Environment **6**:547-553.

Sharda, V. N., P. Samraj, J. S. Samra, and V. Lakshmanan. 1998. Hydrological behaviour of first generation coppiced bluegum plantations in the Nilgiri sub-watersheds. Journal of Hydrology **211**:50-60.

Shirato, Y., I. Taniyama, and T. H. Zhang. 2004. Changes in soil properties after afforestation in Horqin Sandy Land, North China. Soil Science and Plant Nutrition **50**:537-543.

Siemann, E., and W. E. Rogers. 2003. Changes in light and nitrogen availability under pioneer trees may indirectly facilitate tree invasions of grasslands. Journal of Ecology **91**:923-931.

Soriano, A., R. J. C. Leon, O. E. Sala, R. S. Lavado, V. A. Deregibus, M. A. S. O. A. V. C. A. Cauhepe, and J. H. Lemcoff. 1991. Rio de la Plata Grasslands. Pages 367-407 *in* R. T. Coupland editor. Natural Grasslands: Introduction and Western Hemisphere. Elsevier, Amsterdam.

Spiegelberger, T., D. Matthies, H. Muller-Scharer, and U. Schaffer. 2006. Scale-dependent effects of land use on plant species richness of mountain grassland in the European Alps. Ecography **29**:541-548.

Steinauer, E. M., and S. L. Collins. 1995. Effects of urine deposition on small-scale patch structure in prairie vegetation. Ecology **76**:1195-1205.

Texeira, M., and A. Altesor. 2009. Small-scale spatial dynamics of vegetation in a grazed Uruguayan grassland. Austral Ecology **34**:386-394.

Tuomisto, H., K. Ruokolainen, and M. Yli-Halla. 2003. Dispersal, environment, and floristic variation of western Amazonian forests. Science **299**:241-244.

Uruguay XXI: Investment and Export Promotion Agency. Forestry industry: investment opportunities in Uruguay. 1-28. 2010. Montevideo, Republica Oriental del Uruguay.

Valone, T. J. 2003. Examination of interaction effects of multiple disturbances on an arid plant community. Southwestern Naturalist **48**:481-490.

Van Auken, O. W. 2000. Shrub invasion of North American semiarid grasslands. Annual Review of Ecology and Systematics **31**:197-215.

van der Heijden, M. G. A., R. D. Bardgett, and N. M. van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters **11**:296-310.

Vega, E., G. Baldi, E. G. Jobbagy, and J. M. Paruelo. 2009. Land use change patterns in the Rio de la Plata grasslands: the influence of phytogeographric and political boundaries. Agriculture Ecosystems & Environment **134**:287-292.

Venkatesh, B., N. Lakshman, B. K. Purandara, and V. B. Reddy. 2011. Analysis of observed soil moisture patterns under different land covers in Western Ghats, India. Journal of Hydrology **397**:281-294.

von Stackelberg, N. O., G. M. Chescheir, R. W. Skaggs, and D. M. Amatya. 2007. Simulation of the hydrologic effects of afforestation in the Tacuarembo River basin, Uruguay. Transactions of the ASAE **50**:455-468.

Walter H. 1971. Ecology of tropical and subtropical vegetation. Oliver and Boyd, Edinburgh.

Walter H. 1973. Vegetation of the earth in relation to climate and the eco-physiological conditions., 2nd edition. English Universities Press, London.

Wayman, R. B., and M. North. 2007. Initial response of a mixed-conifer understory plant community to burning and thinning restoration treatments. Forest Ecology and Management **239**:32-44.

Wen, Y., D. Ye, F. Chen, S. Liu, and H. Liang. 2010. The changes of understory plant diversity in continuous cropping system of Eucalyptus plantations, South China. Journal of Forest Research **15**:252-258.

WFO. Uruguay: Developing Forest Sector. 1-9. 2002. Portland, World Forestry Center. A WFI Market Brief Series.

Wilby, A., and V. K. Brown. 2001. Herbivory, litter and soil disturbance as determinants of vegetation dynamics during early old-field succession under set-aside. Oecologia **127**:259-265.

Zar J. H. 1999. Biostatistical Analysis., 4th edition. Pearson Education, Inc., Upper Saddle River.

Zuloaga F.O., Morrone O. & Belgrano M.J. Catalogo de las Plantas Vasculares del Cono Sur. [1-3], 1-3348. 2008. St. Louis, Missouri Botanical Garden press.

APPENDIX A

Site photographs for each agroforestry management phase. Photographs are labeled by phase. All photographs are taken by the author, unless otherwise noted.

Grassland, grazed by cattle (as well as horses).

Young Forest, with grazing excluded since trees were planted; photo by Cat Adams.

Mid-stage Forest, with grazing.

Old Forest, with grazing, and following thinning.

Post-Harvest, on average, six months following harvest, with continued grazing.

APPENDIX B

Mean species richness by life form trait category and agroforestry management phase.

	Grassland	Young Forest	Mid-stage Forest	Old Forest	Post-Harvest
Annual graminoid	1.4	2.0	1.0	1.8	1.8
Annual herb	5.4	8.4	1.7	6.0	6.5
Fern	1.0	0.0	1.0	1.5	1.5
Perennial graminoid	15.6	13.2	4.4	8.8	8.0
Perennial herb	25.6	29.2	11.4	13.2	15.0
Woody	5.8	4.2	2.8	3.8	5.0
Unknown	13.0	10.6	4.9	5.7	4.0
Total	67.8	67.6	27.2	40.8	41.8

APPENDIX C

Common families by phase, in order of average species count (means species/site). Totals were calculated as the mean number of species between sites within each agroforestry management phase.

Grassland		Young Forest		Mid-stage Forest		Old Forest		Post-Harvest	
Poaceae	17.2	Asteraceae	14.6	*unknown*	5.2	Asteraceae	7.4	Asteraceae	10
Asteraceae	12.6	Poaceae	14.6	Poaceae	4.6	Poaceae	7.2	Poaceae	7.4
unknown	8.8	Cyperaceae	5.8	Asteraceae	2.4	*unknown*	5.6	*unknown*	5.4
Cyperaceae	5.8	*unknown*	5.6	Cyperaceae	2.4	Cyperaceae	4.8	Cyperaceae	3.8
Rubiaceae	2.8	Rubiaceae	3	Fabaceae	1.4	Juncaceae	2	Apiaceae	1.8
Apiaceae	2.4	Apiaceae	2.8	Oxalidaceae	1.4	Apiaceae	1.2	Juncaceae	1.6
Oxalidaceae	2.4	Fabaceae	2.4	Juncaceae	1	Rubiaceae	1.2	Solanaceae	1.4
Juncaceae	2.2	Juncaceae	1.8	Commelinaceae	0.8	Solanaceae	1.2	Oxalidaceae	1
Fabaceae	1.6	Oxalidaceae	1.8	Lamiaceae	0.8	Commelinaceae	0.8	Rubiaceae	1
Convulvulaceae	1	Lamiaceae	1.2	Solanaceae	0.8	Oxalidaceae	0.8	Commelinaceae	0.8
Iridaceae	1	Solanaceae	1.2	Iridaceae	0.6	Pteridaceae	0.8	Convulvulaceae	0.8
Lamiaceae	1	Caryophyllaceae	1	Pteridaceae	0.6	Convulvulaceae	0.6	Fabaceae	0.8
Plantaginaceae	1	Convulvulaceae	1	Rubiaceae	0.6	Fabaceae	0.6	Lamiaceae	0.8
Amaranthaceae	0.8	Plantaginaceae	1	Apiaceae	0.4	Lamiaceae	0.6	Malvaceae	0.8
Hypoxidaceae	0.8	Amaranthaceae	0.8	Convulvulaceae	0.4	Malvaceae	0.6	Plantaginaceae	0.8
Malvaceae	0.8	Campanulaceae	0.8	Hypoxidaceae	0.4	Amaranthaceae	0.4	Amaranthaceae	0.4
Commelinaceae	0.6	Iridaceae	0.8	Liliaceae	0.4	Campanulaceae	0.4	Caryophyllaceae	0.4
Pteridaceae	0.6	Verbenaceae	0.8	Lythraceae	0.4	Caryophyllaceae	0.4	Pteridaceae	0.4
Turneraceae	0.6	Cistaceae	0.6	Malvaceae	0.4	Iridaceae	0.4	Buddlegaceae	0.2
Verbenaceae	0.6	Commelinaceae	0.6	Turneraceae	0.4	Liliaceae	0.4	Campanulaceae	0.2
Cistaceae	0.4	Hypoxidaceae	0.6	Amaranthaceae	0.2	Plantaginaceae	0.4	Iridaceae	0.2
Liliaceae	0.4	Lythraceae	0.6	Caryophyllaceae	0.2	Thelypteridaceae	0.4	Liliaceae	0.2
Lythraceae	0.4	Polygalaceae	0.6	Moraceae	0.2	Turneraceae	0.4	Molluginaceae	0.2
Polygalaceae	0.4	Scrophulariaceae	0.6	Myrsinaceae	0.2	Urticaceae	0.4	Moraceae	0.2
Scrophulariaceae	0.4	Turneraceae	0.6	Myrtaceae	0.2	Verbenaceae	0.4	Myrtaceae	0.2
Acanthaceae	0.2	Euphorbiaceae	0.4	Pinaceae	0.2	Acanthaceae	0.2	Polygalaceae	0.2
Amaryllidaceae	0.2	Malvaceae	0.4	Polygonaceae	0.2	Hypoxidaceae	0.2	Primulaceae	0.2
Buddlegaceae	0.2	Acanthaceae	0.2	Urticaceae	0.2	Melastomataceae	0.2	Scrophulariaceae	0.2
Euphorbiaceae	0.2	Amaranthaceeae	0.2	Verbenaceae	0.2	Moraceae	0.2	Turneraceae	0.2
Melastomataceae	0.2	Liliaceae	0.2			Pinaceae	0.2	Urticaceae	0.2
Primulaceae	0.2	Melastomataceae	0.2			Polygonaceae	0.2		
		Molluginaceae	0.2			Primulaceae	0.2		
		Polygonaceae	0.2						
		Primulaceae	0.2						
		Ranunculaceae	0.2						
Total	67.8	Total	67.6	Total	27.2	Total	40.8	Total	41.8

Appendix D

Site photographs of grazed forest and grasslands and ungrazed forest and grasslands (inside exclosures).

Forest study site inside (left) and outside (right) exclosures, at time of study establishment (November 2009).

Grassland study site outside (far left) and inside exclosures, at time of study establishment (November 2009).

Study site in grazed (left) and ungrazed) (right) forest (background) and grassland (foreground), 6 months after exclosure establishment.

Study site in grazed (left) and ungrazed (right) grassland, 12 months after exclosure establishment.

Study site in ungrazed forest, 12 months after exclosure establishment. Photo by Elise Koncsek.

Study site in grazed forest, 12 months after exclosure establishment.

Study site in grazed (left) and ungrazed (right) grassland, 12 months after exclosure establishment.

Study site in grazed (left) and ungrazed forest (right), 18 months after exclosures were established.

Study site in grazed (left) and ungrazed (right) grassland, near the forest edge (foreground), 18 months after exclosures were established.

VITA

Laura Six was born in Chehalis, Washington and currently calls Bonney Lake, Washington, home. She graduated with a Bachelor of Science in Conservation of Wildland Resources and a Master of Science in Forest Resources from the University of Washington. She works as a Plant Ecologist for Weyerhaeuser NR Global Timberlands Technology. In 2012, she earned a Doctor of Philosophy at the University of Washington in Forest and Environmental Sciences.