UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

Tese de Doutorado

Restauração Ecológica nos Campos Sulinos: diagnóstico de degradação, superação de filtros bióticos e abióticos e reflexões sobre sistemas campestres

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Orientador: Dr. Gerhard Ernst Overbeck

Porto Alegre, 08 de setembro de 2020

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Botânica da Universidade Federal do Rio Grande do Sul como parte dos requisitos para obtenção do título de Doutora em Botânica com ênfase em Ecologia Vegetal.

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Todo pasa y todo queda,
pero lo nuestro es pasar,
pasar haciendo caminos,
caminos sobre el mar.

Nunca perseguí la gloria,
ni dejar en la memoria
de los hombres mi canción;
yo amo los mundos sutiles,
ingrávidos y gentiles,
como pompas de jabón.

Me gusta verlos pintarse
de sol y grana, volar
bajo el cielo azul, temblar
súbitamente y quebrarse...

Nunca perseguí la gloria.

Caminante, son tus huellas
el camino y nada más;
caminante, no hay camino,
se hace camino al andar.

Al andar se hace camino
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.

Caminante no hay camino
sino estelas en la mar...

Antonio Machado

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Resumo

A conversão de áreas naturais vem crescendo a passos largos em âmbito mundial e, junto com esta tendência, a pesquisa em restauração ecológica se expande, buscando compreender processos que influenciam a montagem de comunidades e validar conceitos, possibilitado aliar teoria e prática na recuperação de ambientes degradados. Para aumentar as chances de sucesso de um projeto de restauração, é fundamental diagnosticar quais são os filtros bióticos e abióticos presentes na área degradada para, a partir disto, elaborar técnicas que proporcionem a superação destes fatores limitantes. No Rio Grande do Sul, a silvicultura é uma das formas de uso do solo que mais se expande sobre áreas naturais, porém, não existe até o momento experiências de pesquisas de restauração ecológica em áreas originalmente campestres convertidas em silvicultura. Neste contexto, esta tese visou contribuir com o conhecimento dos principais filtros bióticos e abióticos atuantes em campos degradados pelo plantio de *Pinus sp*. nos Campos de Cima da Serra e testar técnicas de restauração para superar estes filtros. A tese foi estruturada em três capítulos com propósitos complementares. No primeiro capítulo foi realizado um diagnóstico do impacto de 25 anos de conversão de um campo natural para um plantio de *Pinus* nos Campos de Cima da Serra. Nossos resultados mostram um banco de sementes considerávelmente reduzido em densidade e riqueza de espécies (limitação biótica), apontando para uma regeneração natural limitada e distinta em composição de espécies. O segundo capítulo constitui de um experimento de métodos para restauração, com intuito de superar as barreiras ambientais resultantes da degradação. Os resultados deste capítulo mostram que intervenções para superação da escassez de sementes, como a introdução de feno, e o preparo do substrato que receberá o feno são fundamentais para a introdução de espécies-alvo. O terceiro capítulo junta conceitos e experiências para discutir temas importantes para o avanço da restauração de ecossistemas campestres, com o intuito de levantar uma discussão importante para políticas públicas ambientais e para a sociedade em geral. O conteúdo apresentado nesta tese auxilia na compreensão dos filtros ambientais atuantes em áreas degradas por silvicultura nos Campos de Cima da Serra e de conceitos envolvidos na restauração de ecossistemas campestres.

Palavras-chave*:* filtros ambientais; degradação; silvicultura; banco de sementes; limitação de sementes

Abstract

The conversion of natural areas has been growing at a global pace and, together with this trend, ecological restoration research has expanded, seeking to understand processes that influence the assembly of communities and validate concepts, making it possible to combine theory and practice in the recovery of degraded environments. To increase the chances of success of a restoration project, it is essential to diagnose which are the biotic and abiotic filters present in the degraded area, from this, develop techniques providing overcoming these limiting factors. In the state of Rio Grande do Sul, silviculture is one of the most widespread forms of land use on natural areas. However, there are no ecological restoration research experiences in originally grasslands areas converted to silviculture. In this context, this thesis aimed to contribute to the knowledge of the main environmental filters working in degraded grasslans by Pine plantation in Campos de Cima da Serra and test restoration techniques to overcome these filters. The thesis was structured in three chapters with complementary purposes. In the first chapter a diagnosis was made of the impact of 25 years of conversion of a natural field to *Pinus* planting in the Campos de Cima da Serra. The second chapter consists of an experiment in methods for restoration, to overcome the environmental barriers resulting from degradation. The third chapter brings together concepts and experiences to discuss important themes for the advancement of the restoration of grasslands ecosystems, to raise an important discussion for environmental public policies and for society in general. The content presented in this thesis assists in the understanding of environmental filters working in areas degraded by silviculture in the Campos de Cima da Serra and concepts involved in the restoration of grasslands.

Key-words*: environmental filters; degradation; afforestry; seed bank; seed limitation*

Sumário

INTRODUÇÃO GERAL

A ecologia da restauração vem aumentando sua visibilidade mundialmente nos últimos anos e a crescente sensibilização da sociedade pelo tema é decorrente, principalmente, de experiências negativas que vêm sendo vividas em consequência da conversão desenfreada de áreas naturais. Alterações nos serviços ecossistêmicos, perda de biodiversidade, contaminação de recursos hídricos, erosões e alagamentos são algumas das consequências, cada vez mais frequentes, vividas pela sociedade (Brown & Macleod, 2017). Políticas públicas mais restritivas que impeçam novas áreas de sofrerem conversão, são ferramentas importantes para conservação, porém em muitos casos, dependendo do ecossistema, além de proteger os atuais remanescentes é preciso também recuperar áreas já degradas para a proteger a biodiversidade e serviços ecossistêmicos. No atual cenário global, mudanças ambientais e socioeconômicas tem consequências tanto na conversão contínua de áreas naturais para uso intenso do solo quanto no abandono de áreas já convertidas (Cramer, Hobbs, & Standish, 2008). Diferentes iniciativas vêm sendo firmadas com o intuito de frear os impactos antrópicos na natureza e atingir metas internacionais para conservação e recuperação de áreas degradadas. A *Global Restoration Iniciative, Aichi Biodiversity Targets* e *Iniciative 20x20* são exemplos que mostram a preocupação e o comprometimento de diversos países em relação à recuperação da vegetação nativa.

No âmbito nacional, as políticas ambientais vêm aumentando suas exigências quanto à responsabilização de infratores de crimes ambientais e quanto às ações necessárias para a recuperação das áreas degradadas. A nova Lei de Proteção da Vegetação Nativa (lei n° 12.651/2012) responsabiliza proprietários que tenham suprimido a vegetação nativa em áreas de proteção permanente (APP) e reserva legal (RL) de recuperarem a vegetação originária. Também, a restauração de áreas degradadas foi incluída como um dos princípios da Política Nacional do Meio Ambiente (lei n° 6.938/1981) e, novas políticas ambientais como a Política Nacional para Recuperação da Vegetação Nativa (Proveg) vem sendo elaboradas, no âmbito da restauração ambiental com o intuito de promover, articular e integrar ações que promovam a recuperação da vegetação nativa. Estimativas recentes indicam que existem aproximadamente 21 milhões de hectares a serem restaurados em áreas de

Preservação Permanente e Reserva Legal (Soares-Filho et al., 2014), e as metas são para que até 2030 12 milhões hectares estejam restaurados no país. Como ferramenta de apoio aos proprietários e para órgãos públicos para atender às novas demandas de restauração foi desenvolvido pelo governo em colaboração com diversas instituições o Plano Nacional de Recuperação da Vegetação Nativa (Ministério do Meio Ambiente 2017) onde foram desenvolvidas estratégias para dar suporte às exigências legais de restauração. Contudo, o caminho para a restauração de áreas degradadas permanece com lacunas de conhecimento científico a serem preenchidas que são fundamentais para darem suporte e validação às ações práticas de restauração, principalmente quando se trata de estudos sobre restauração em ecossistemas campestres, onde as experiências existentes até o momento são escassas, apesar das altas taxas de conversão de campos naturais em áreas de agricultura e silvicultura (Cordeiro & Hasenack 2009) e, apesar também da presença de extensas áreas degradadas dentro ou no entorno de Unidades de Conservação (Gerhard E Overbeck et al., 2013).

Existem dois tipos principais de transformação da paisagem campestre atualmente no Rio Grande do Sul, o estabelecimento de lavouras e de áreas de silvicultura. Existem diferenças claras no desenvolvimento espontâneo da vegetação após abandono quando comparado com áreas campestres bem conservadas (áreas de referência). Em situação de abandono após lavoura e silvicultura, a vegetação que se estabelece no local difere consideravelmente da vegetação campestre característica e há o predomínio de espécies ruderais, exóticas ou arbustos (Bonilha et al., 2017; Koch et al., 2016; Torchelsen, Cadenazzi, & Overbeck, 2018), o que indica que a vegetação original não consegue se restabelecer sem assistência, ou seja, sem ação de restauração ativa. No caso de silvicultura de *Pinus*, a presença de acículas e galhos muitas vezes dificultam a regeneração da vegetação nativa em grandes áreas por períodos consideráveis.

Dependendo do tipo de degradação ocorrente, diferentes filtros (barreiras) podem influenciar durante o processo de restauração. Neste contexto que é possível trabalhar na prática através da estreita ligação entre a ecologia de restauração e a teoria ecológica, visto que os processos envolvidos durante a restauração podem ser vistos como processos de montagem de comunidades (Temperton & Hobbs 2004)

Se houve mudanças fortes na disponibilidade, estrutura ou composição do substrato, por exemplo, após processos de erosão ou acúmulo expressivo de biomassa morta, a regeneração da vegetação pode ser limitada por filtros abióticos que impedem o estabelecimento das plantas. Além disto, existem filtros bióticos como por exemplo, limitações devido à falta de propágulos das espécies-alvo no sistema a ser restaurado. No caso da vegetação campestre, o potencial de dispersão geralmente é baixo, o que impossibilita a recuperação não-assistida e pode constituir o principal desafio em projetos de restauração (Öster, Ask, Cousins, & Eriksson, 2009). Para os Campos sulinos, estudos mostram que o banco de sementes de espécies campestres nativas tem riqueza fortemente reduzida após períodos relativamente curtos de uso agrícola (Garcia 2009, Vieira et al. 2015).

Em consequência destes filtros bióticos, técnicas de introdução de sementes são de alta importância para projetos de restauração em ecossistemas campestres (Kiehl, Kirmer, Donath, Rasran, & Hölzel, 2010). Entre as técnicas mais aplicadas na restauração de campos em diversas regiões do mundo, está a semeadura direta de espécies-alvo, a transposição de feno (*hay transfer)* (Baasch, Kirmer, & Tischew, 2012; Coiffait-Gombault, Buisson, & Dutoit, 2011; Kiehl & Wagner, 2006) e o transplante de leivas (*turf transplant*) (Aradottir, 2012; Bay & Ebersole, 2006). A semeadura direta, geralmente com mistura de espécies nativas (Kiehl, 2010) depende da disponibilidade de sementes das espécies-alvo no mercado. No caso dos Campos Sulinos, atualmente é impossível obter comercialmente sementes de espécies nativas, o que é um limitante para projetos de restauração em grandes escalas. Como alternativa à falta de sementes no mercado, a transposição de feno pode ser promissora e um método com baixo custo que consiste em cortar a biomassa aérea de plantas em frutificação em locais com vegetação campestre bem conservada e a consequente transposição deste material para as áreas degradadas. Esta técnica vem sendo utilizada com sucesso em diferentes regiões do mundo, sendo que o feno contribui também para condições microclimáticas favoráveis no local e possibilita a transposição de um número maior de espécies de forma simultânea e preserva características genéticas das populações localmente adaptadas (Hölzel & Otte, 2003; Kiehl, 2010). O transplante de leivas é método que apresenta sucesso considerável na introdução de espécies-alvo para estudos na Europa (Kiehl et al. 2010) no entanto, esta técnica tem a desvantagem de causar impacto no local onde as leivas foram removidas.

Até o momento existem poucas experiencias (Thomas et al. 2018, Prado et al. 2018) sobre quais técnicas são as mais adequadas para a restauração ecológica dos Campos sulinos e quais adaptações de técnicas desenvolvidas em outras regiões seriam necessárias, como por exemplo, para contemplar a coexistência de espécies hibernais e estivais.

Esta tese teve como objetivo geral contribuir com o conhecimento dos diferentes filtros ambientais atuantes no processo de restauração de campos degradados por plantio de *Pinus sp*. nos Campos de Cima da Serra, quais técnicas apresentam resultados mais adequados para superar estes filtros e contextualizar fatores que regem a dinâmica dos campos em ações para restauração.

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CAPÍTULO 1

Small grassland seed bank in South Brazilian highland grasslands further reduced by silviculture

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Small grassland seed bank in grasslands and tree plantations in former grassland sites in the South Brazilian highlands

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Abstract

The soil seed bank can be an important source for vegetation regeneration, and data on the similarity between aboveground vegetation and the seed bank can provide information about successional pathways after disturbances or land-use change. We conducted this study in natural grasslands in the subtropical highland region in southern Brazil. We evaluated the effect of silviculture on richness, density, and composition of the seed bank at former grassland sites converted to pine plantations 25 years ago. We worked at six grassland sites and three pine plantation sites and used the seedling emergence method. Seed bank density and richness in grasslands was lower than those reported in similar environments in other regions. Species richness and density varied considerably within each vegetation type therefore, richness and density were not statistically significant, while composition varied among vegetation types. In terms of species, the pine plantation seed bank was a small subset of the grassland seed bank. Seeds of typical grassland species were missing in the pine plantation, but also had only low abundances in the grassland, and similarity of seed bank and vegetation was low (less than 20%). The low seed density found in this study, including in grasslands areas, indicates that regeneration of species from the soil seed bank likely is of a limited role for the maintenance of plant populations after disturbances in this system. Our data further suggest that natural regeneration after tree planting in grasslands is reduced due to seed limitation.

Keywords: Pine plantation; Atlantic Forest; Seed limitation; Restoration

INTRODUCTION

Tree plantations currently cover about 264 million ha of the planet, with an annual increase of 5 million ha (data from 2000-2010; FAO 2010). In developing countries, tree plantations are one of the main forms of land-use (Zhang et al. 2014), and in many cases, policy stimulates their expansion. This includes tree planting where they did not occur historically (afforestation), principally using species with high commercial value. Carbon sequestration is often used as an argument in favor of tree plantings; however, there are negative effects on other ecosystem processes and services. Among the consequence of tree plantings in regions where non-forest ecosystems dominate are loss of habitat and disruption or changes of biological processes such as nutrient cycling (Berthrong et al. 2009), hydrological cycles (Jackson et al. 2005) as well as changes in biodiversity (Bremer & Farley 2010).

In southeastern South America, the expansion of tree monocultures, principally of *Pinus spp*. and *Eucalyptus spp*., started in the late 1980s (Gautreau & Velez 2011). In South Brazil, pine is planted principally in the highland grassland region located at the southern tip of the Atlantic Forest domain. Considered old-growth grasslands (*sensu* Veldman et al. 2015), these grasslands present high endemism levels (25% of the original flora; Iganci *et al.* 2011), and high plant diversity (Andrade et al. 2016). They are traditionally used for extensive livestock grazing with rather low stocking rates, and disturbances such as fire, often used as a management tool, and grazing are responsible for maintenance of the grasslands and their biodiversity (e.g. Andrade et al. 2015).

In the past 25 years, public policies have stimulated the planting of exotic tree species in the region, although several pine species are widely known as invasive (Gautreau & Vélez, 2011). Hermann et al. (2016) assessed land-use changes in part of the highland region where our study was conducted: their study revealed an expansion of 94% in silviculture occupation in the period from 2003 to 2009. However, either for economic reasons or due to legal requirements (e.g. planting had been conducted in areas with restrictions due to conservation purposes), some of these areas are abandoned after clear-cutting, including areas in or close to protected areas. The remaining flora in the soil as well the paths of the regeneration trajectory of these grasslands converted into silviculture remain unknown. Studies on restoration techniques in the South Brazilian region are recent and few (Overbeck et al. 2013, Overbeck & Müller 2017, Thomas et al. 2019a, b) and consequently, little data is available on restoration success, or even potential for spontaneous recovery.

At plantation sites, shading by trees, along with other changes, e.g. in soil properties, over several years leads to virtually complete suppression of local plant communities (Galloway, Holmes, Gaertner, & Esler, 2017). Only a small number of species can persist over time under these conditions. Natural recovery of vegetation after clear cutting, at the end of use of the area as plantation, depends on the soil seed bank and on the dispersal of native species into the degraded area, in interaction with abiotic factors, such as soil properties (Torchelsen et al., 2018). Potentially, the seed bank can be an important source for vegetation regeneration and may play a key role in the assembly process of the community (Marteinsdóttir 2014). Data on the similarity between aboveground vegetation and the seed bank can provide information about successional pathways after abandonment (Loydi et al. 2012) and can serve as a prognostic tool to infer the early stages of colonization and to assist in planning actions for restoration. The available studies on the seed bank of subtropical grasslands in South America (e.g. Maia et al. 2003, Favreto & Medeiros 2006, Haretche & Rodríguez 2006, Vieira et al. 2015, Lipoma et. al. 2018) indicate, in general, the presence of large seed banks in both primary and secondary grasslands. They also indicate a clear pattern of dominance of ruderal and annual species in areas with a history of intensive land-use, generating differences in composition with preserved grassland areas. However, no studies exist so far for the highland grasslands of the Atlantic forest domain of southern Brazil, which are different from Pampa grasslands in terms of climate, soil, and species composition (Andrade et al. 2019). Also, the effect of tree plantations on the seed bank of grassland has also not been evaluated so far for South American subtropical grasslands in general, and studies in other tropical and subtropical grassland regions around the world still are scarce (e.g. Galloway et al. 2017).

Here we evaluate the soil seed bank of natural subtropical grasslands as well as that of former grassland sites now under pine plantations. Our study thus aims to contribute to the knowledge on dynamics of grassland systems in the region and to a better understanding of the effect of pine plantations on the soil seed bank and thus post-plantation vegetation recovery. Specifically, we (I) characterize, for the first time, the seed bank of natural grasslands in the South Brazilian highland grassland region in terms of richness, density, and composition; (II) evaluate the effect of tree plantation on richness, density, and composition of the seed bank in converted grassland area, in comparison with not converted grassland, and (III) relate the seed bank composition in tree plantations and natural grasslands areas to aboveground grassland vegetation and discuss the potential contribution of the seed bank for vegetation recovery.

METHODS

Study area

Our study sites are in the highland grassland region in the southern part of Brazil's Atlantic Forest domain (29°04'12'' S, 50°00'49'' W). Regional climate is Cfb according to Köppen climate classification, and altitude approximately 1000 m. Mean annual temperature is 15°C and mean annual precipitation is 1881 mm (climate-data.org). The region is a plateau formed by basalt, rhyolite and rhyodacit rocks of Serra Geral formation. Soils are classified as Cambisoils according to FAO, 1997 (Cambissolos in the Brazilian classification; Embrapa 2013). Natural vegetation in the region is composed of mosaics of *Araucaria* forest, cloud forest and grasslands (Leite & Klein 1990). These highland grasslands have been used for livestock grazing since European colonization. However, the presence of large herbivores – today extinct – even before the arrival of native American people is confirmed by the fossil record in the region (Scherer et al. 2007). Based on charcoal records from peat bogs, we know that fire has been rare during the Glacial maximum but became more frequent at the beginning of the Holocene (Behling & Pillar 2006). Today, fire, usually every other year, is used as a management tool to remove accumulated biomass to stimulate young leaf regrowth after winter. In terms of their floristic composition, the highland grasslands are dominated by C4 tussock grasses such as *Andropogon lateralis* Nees, *Sorgastrum scaberrimum* (Nees) Herter, *Axonopus pellitus* (Nees ex Trin.) Hitchc. & Chase and a high representation of Fabaceae family (Andrade et al. 2019). The region encompasses two important national parks, Aparados da Serra and Serra Geral, and other state and private protected areas. In the region, we find vast areas of pine plantations, with single planting cycles of 30 years on average, causing loss and fragmentation of natural areas (Hermann et al. 2016).

For this study, we chose six well conserved grasslands, four located in Serra Geral National Park and two in Aparados da Serra National Park (Fig.1), and three pine plantations established in former grasslands areas. Two of them were in the buffer zone of the National parks, and one of them at the edge of the park. Pine plantations were initiated about 25 years ago. Sites were situated in three blocks, each with one pine plantation and two natural grassland areas, with the same history and similar floristic composition of grasslands. Distance of blocks varied from 2 to 20 km, and areas within each block had distances of 500 to 2000 m (see Fig. 1 for scheme of study design).

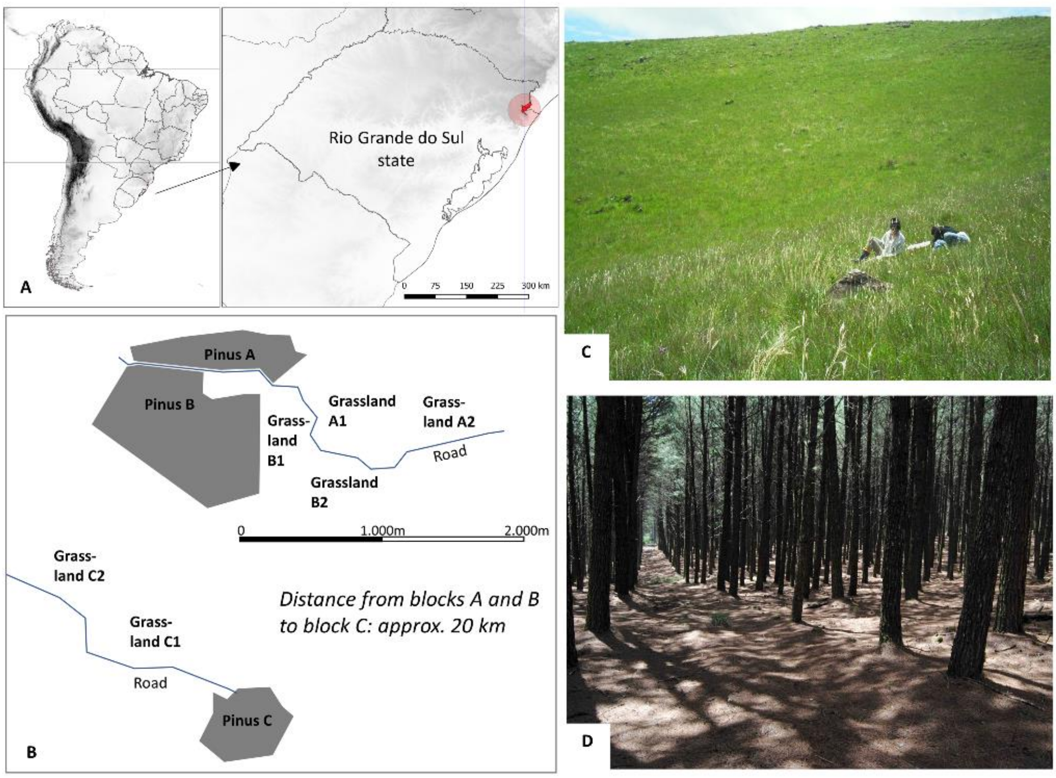

Figure 1. Location of the study areas. **A**. Location of National Parks Aparados da Serra and Serra Geral in Brazil and Rio Grande do Sul state. **B.** Schematic representation of the study sites. **C.** Picture of one of the natural grasslands. **D**. Picture of one of the pine plantations.

Vegetation sampling

Quantitative vegetation sampling at the grassland sites was conducted in December 2014, in 10 plots of 1 m², randomly allocated, per grassland area. Distance between plots was approx. 50 m. Cover of all vascular species was recorded using the Londo decimal scale (Londo, 1976). In the pine plantation areas, no vegetation survey was conducted, as ground layer vegetation was completely absent.

Seed bank sampling and assessment

The seed bank study was carried out using the seedling emergence method, which evaluates only the viable seeds in the soil (Thompson & Grime 1979). Soil samples for the seed bank study were collected in grasslands and current pine plantations. Samples were collected in two seasons (spring and autumn) with the intention of accessing both the transient seed bank and persistent seed bank (Thompson & Grime 1979). We used five sampling points in each study area, totaling 30 samples from grassland and 15 from pine plantations (five per area). Distances between sampling points were approx. 50 m. Soil samples were collected with an auger (diameter: 5 cm; depth: 10 cm). At each sample point we collected four sub-samples which were mixed, resulting in one composite sample per point. All sample points were randomly selected.

For seedling emergence, we used 50% of the soil collected in the field. Soil was mixed with vermiculite (50:50), to maintain humidity, and spread in trays (soil depth: 2-3 cm). Samples were kept in a greenhouse with irrigation for one year and were monitored weekly. Trays with sterilized soil were distributed among the soil samples from the grasslands to control possible contaminations by plants dispersed close to the experimental facilities. Emerging seedlings were identified, counted, and removed as soon as possible. For species that could not be identified right away, at least one specimen was transplanted into a larger container for development of the reproductive phase, for later identification. Most taxa (83%) were identified to the species level and 92% to the genera level. Some individuals died in the trays or transplanted pots before identification was possible, or there was little development of individuals impeding identification.

Data analysis

Data of seeds per sampling point unit were converted to density (seeds per square meter) with the aim of facilitating comparison with other studies. We averaged seed density data from the two seasons together for each sampling point. For statistical analysis, mean values of each studied area were considered, resulting in six average values for the grassland areas and three average values for the Pinus areas. For all analyses, we used randomization tests, with 10.000 iterations. This method (also referred to as permutation test), based on resampling, is also adequate for multivariate data sets, such as compositional data, and has been proposed specifically for vegetation data (details in Pillar & Orlóci 1996). Another advantage is that it does not require normal distribution of data, while preventing robust test results (Pillar & Orlóci 1996); this also makes the method especially appropriate for our data set. For analysis of richness and density data, we used Euclidean distance as dissimilarity measure and for analysis of the seed bank composition chord distance as dissimilarity measure. We analyzed composition similarities among pine plantations soil seed bank, grassland seed bank and aboveground vegetation on grassland areas with Sørensen's Index (2a/2a + b + c), where a = number of species common to both seed banks, b = number of species unique to the first seed bank, and c = number of species unique to the second seed bank, considering all the data set of the seed bank (two seasons). Principal Coordinate Analysis was conducted to visualize difference in seed bank composition between the grasslands and pine plantations, using chord distance as the similarity measure. For all analyses, we used the software MULTIV (Pillar, 2006). We used alpha = 0.05 as significance level.

RESULTS

Above ground vegetation and soil seed bank composition

A total of 178 species were recorded in this study. Of these, 160 species from 31 botanical families were recorded in the established vegetation on natural grasslands. The most abundant species in established vegetation were the grasses *Andropogon lateralis (21%;* cover mean value across sites*)*, *Sorghastrum scaberrimum (12.8%)* and *Axonopus pellitus (7.5%)*, and only two exotic species (*Centella asiatica* and *Paronychia chilensis*), both considered naturalized in the region, were found. Overall, these results indicate a good conservation status of the grasslands (see Table

S1 for complete species list). In the grassland soil seed bank, 45 species from twelve botanical families were recorded. Only 13 species, from five families, were sampled in the seed bank under pine plantation. The main families in established vegetation in the grassland areas were Poaceae (with 68% of the total plant cover), Asteraceae (12%), Cyperaceae (7%), following the general pattern for grasslands of southern Brazil (Boldrini 1997). However, this pattern changed in the seed bank. The grassland seed bank was composed of Poaceae (30% of seed bank density), followed by Cyperaceae (22%), Rubiaceae (20%) and Araliaceae (14%). In the pine plantation, where there was no established herbaceous vegetation, 51% of the seed bank density was by Cyperaceae, followed by Caryophyllaceae (23%), Poaceae (13%) and Rubiaceae (9%). In terms of species richness per area, grassland seed bank and pine plantation seed bank did not differ (p=0.202), presenting an average of 4.4 species/m² (SD 3.7) in grassland seed bank compared to 1.6 species/m² (SD 0.8) in pine seed bank. However, the two seed banks differed in terms of composition (p=0.002), see Table S2.

Principal species likewise differed between established vegetation and both types of the soil seed bank. Tussock grasses principally composed the established vegetation. In contrast, the species with highest density in the grassland seed bank were *Galium humile* (390.5 seeds/m², *Hydrocotile exigua* (339.5 seeds/m²) and *Axonopus pellitus* (178.2 seeds/m²). The most abundant species in the pine seed bank were *Bulbostylis brevifolia* (237.7 seed/m²), *Paronychia chilensis* (203.7 seeds/m²), *Bulbostylis hirtella, Galium humile and Dichanthelium sabulorum* (each with 85 seeds/m²). Mean seed density in the grassland seed bank was 2487 seeds/m² (SD 2246) and only 900 seeds/m² (SD 561) in the pine plantation seed bank, however, the differences were not significant (p=0.241) due to large spatial heterogeneity of the seed bank (Fig. 2, based on individual sampling points to better show this variation; Suppl. Mat 1 for results of statistical analyses).

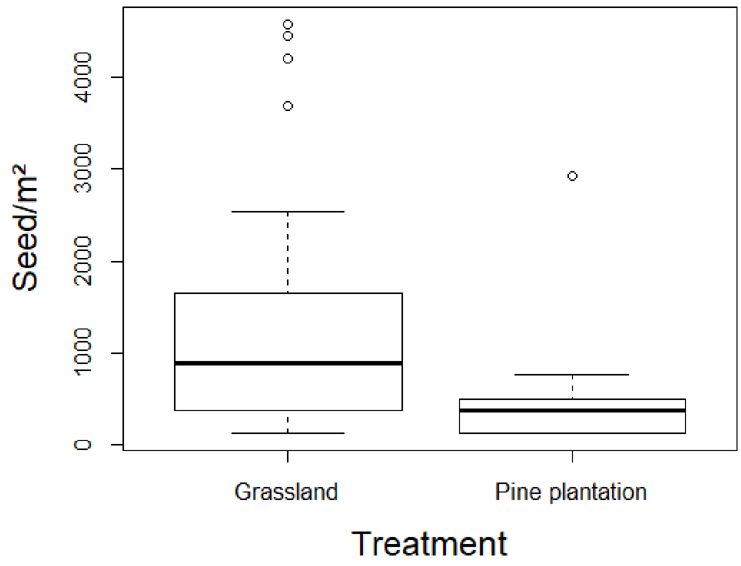

Figure 2: Soil seed density in a natural grassland and under pine plantation in highland grasslands in Rio Grande do Sul, Brazil. To better illustrate the high variability in seed distribution in the figure, we used sampling point data (30 sampling units for natural grassland; 15 for pine plantations), not average values per area (i.e. 5 points per area, as used for statistical analysis). Each box represents the inter-quartile range of the data (25% - 75%). Dark middle bar refers to median, whiskers represent the minimum and maximum values and dot symbols identify outliers.

Similarities among established vegetation and seed banks

In total, only seven species were shared among grassland vegetation, grassland seed bank, and pine plantation seed bank (Fig. 3). Of the 160 species present in the established vegetation, 27 were also recorded in the grassland seed bank, and only eight in the soil seed bank under pine plantations.

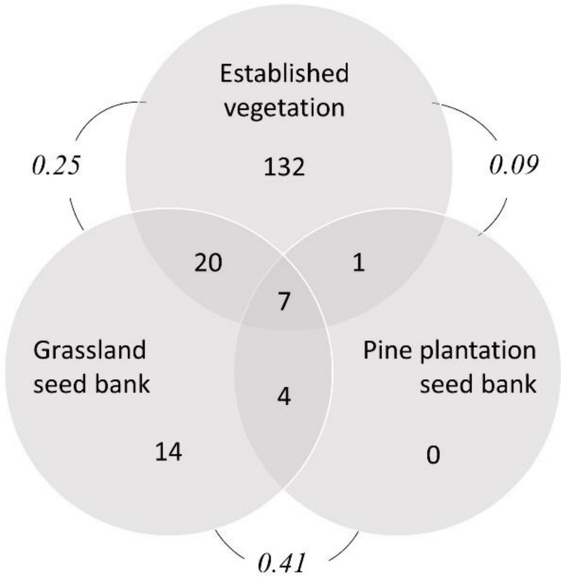

Figure 3. Venn diagram illustrating the number of exclusive and common species among established vegetation, grassland seed bank and pine plantation seed bank. Numbers in italics indicate of the Sørensen similarity index, in grassland and afforestation areas in Cambará do Sul, Rio Grande do Sul, Brazil.

Only one species presents in the soil seed bank of the plantation areas, *Paspalum plicatulum* Michx, was not registered in the soil seed bank of the reference areas. No species were recorded as exclusive in the soil seed bank in the pine plantations area. The Sørensen index showed a rather high similarity between both seed banks (0.41), followed by a lower similarity between the grassland seed bank and established vegetation (0.25) and a very low similarity between established vegetation and pine plantation seed bank (0.09). The Principal Coordinates Analysis using the seed bank data (Fig. 4) did not indicate a clear separation between areas.

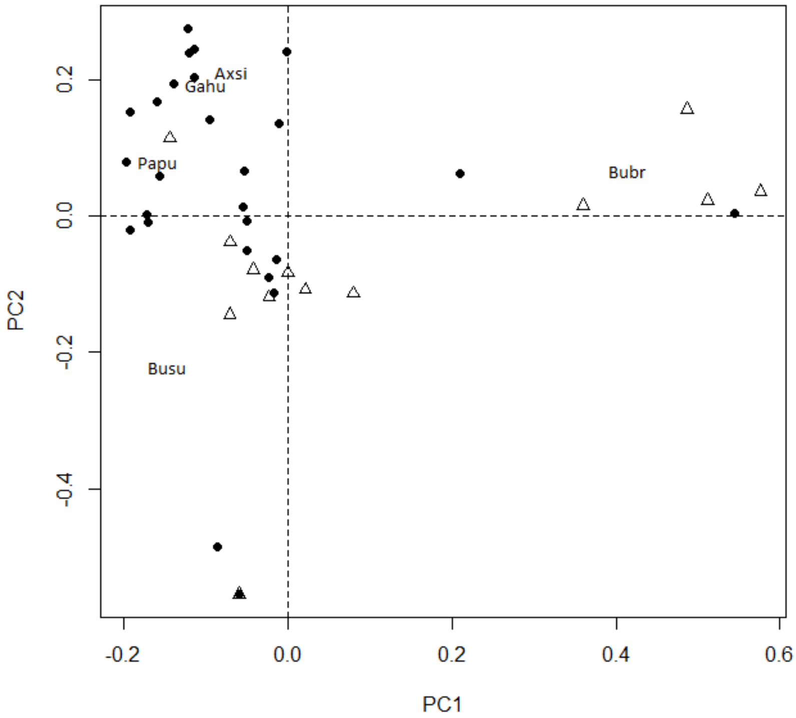

Figure 4. Ordination diagram (Principal Coordinates Analysis) of seed bank composition of natural areas (dots) and pine plantations (triangles) in Cambará, RS, Brazil, based on the data from the sampling points. 4 points in the natural grasslands and 2 points in the pine plantations did not present any germinated seeds, therefore n= 26 and n= 13 for grassland and pine seed bank, respectively. Explanations of the first two ordination axes: 11 % and 9 % respectively. Species correlated with the axes more than 0.2: *Axonopus pellitus*, *Bulbostylis brevifolia* (Bubr), *Bulbostylis subtilis* (Busu), *Galium humile* (Gahu), *Paspalum pumilum* (Papu).

DISCUSSION

Our study characterized, for the first time, the seed bank of South Brazilian highland grasslands in terms of composition and density and investigated the influence of tree plantation on the soil seed bank 25 years after land-use change. While the statistical analysis did not reveal significant differences between grassland and pine plantation sites with respect to seed density and species richness, the composition data clearly indicates that typical grassland species are missing in the pine plantation seed bank that differed significantly from the grassland seed bank in terms of composition. These results differ from those of Galloway *et al.* (2017) in the Fynbos

Biome, where the difference between reference area and 30-year old plantations areas was low. In our study, seeds that persist in soils after a long period of conversion are largely ruderal species with little or no representation in the aboveground vegetation in the highland grasslands of the Atlantic Forest domain of southernmost Brazil. These findings indicate that natural recovery of the typical grassland plant community from the soil seed bank after cutting of trees likely is difficult. Typical and dominant grassland species (target species in restoration) are largely missing, indicating the reduction of regional pool of species in the seed bank, as shown in the Fig. 2. This result is of high relevance from a conservation perspective in a region where silviculture has been introduced over wide areas, without consideration of consequences for biological diversity in a region rich in species and endemism (Hermann et al. 2016) or consideration of possibilities of restoration.

Seed bank richness and density in both types of communities, natural grassland and pine plantation, were low when compared with other seed bank studies that used the seedling emergence method for South American subtropical grasslands (Maia et al. 2003; Haretche & Rodríguez 2006; Vieira et al. 2015; Lipoma et al. 2016). In these studies, density values ranged from 2700 to 59,500 seeds/m² in different soil drainage conditions, with high values under more humid conditions and after land-use changes, i.e. in secondary grasslands. Our study found mean values of 2487 seeds/m² for natural grasslands, even though precipitation in the region is extremely high, which means that conditions are quite humid. While working in one specific region, we analyzed a total of six sites, and our data thus clearly indicates that the seed bank in the highland region has a low seed density per m² when compared to other subtropical grasslands situated further to the South. The same pattern follows for richness, with only 45 recorded species, when compared to the studies cited above, in which the lowest value of richness was 54 species and values reach 122 species.

The average seed density (900 seeds/m²) for former pine plantations was even lower than that found by Bistreau & Mahy (2005) in pine forest on former grassland sites in Belgium, but with a similar 3:1 relation of seed bank density for grassland to forest/plantation as in our study. We recorded the presence of seeds of only 13 species in the afforested area, and many of these with a ruderal character. This, as well as the low representativeness of the Poaceae family, with presence of only two species, evidences the strong effect of afforestation on the grassland soil seed bank. Due to high density of trees in the region, plantations lead to strong shading from trees, litter accumulation, soil compaction, and changes in soil-water availability; conditions that are unfavorable for maintenance of local vegetation (Roig et al. 2005), and, as shown here, also of seeds in the soil. Our study shows that effects go beyond the time of the planting, also acting on the soil seed bank. The thick needle layer in pine plantations not only changes the local microclimate, but also generates a physical barrier, retaining seeds according to their size or shape in the litter layer as reported by Bueno & Baruch (2011). This also limits the input of seeds into the soil, as seeds dispersed from surrounding grasslands areas – if entering the plantation – will hardly be incorporated into the soil seed bank.

Natural grasslands in general show low similarity between seed bank composition and established vegetation (Luzuriaga et al. 2005; Vieira et al. 2015). Nonetheless, the consequences of afforestation on the vegetation is evidenced by even lower values of the Sørensen's index when comparing seed bank with established vegetation, due to both changes in composition and the low richness, as also shown by Bistreau and Mahy (2005) and Zhang et al. (2014). Our results indicate that the pine plantation seed bank contains a small subset of species of the grassland seed bank, composed of species that are tolerant to strong environmental filters, such as changed moisture and shading, and that should have long term persistence. In the afforested areas, the seed bank was mainly composed of species from families that present many species with a ruderal strategy, such as Cyperaceae, i.e. species with the capacity to produce a high number of durable seeds, which together with the low similarity to natural grassland – evaluated through the Sørensen index – indicates that the seed bank does not support the regeneration of the original plant community.

It is known that most grassland species have seeds with short term persistence in the soil or only produce small amounts of viable seeds (Bekker et al. 1998; Maccherini & De Dominicis 2003). In many environments, clonal growth and vegetative reproduction offer ecological advantages, such as high competitiveness and rapid spread (Barrett 2015). In the case of the highland grasslands of the Atlantic Forest biome of southern Brazil, which are composed principally by perennial species and are historically under disturbances as fire and grazing, most species can resprout from above- and belowground buds after disturbance (Overbeck & Pfadenhauer 2007; Fidelis et al. 2014). The importance of these organs for vegetation recovery was also observed by Lipoma et al. (2018) in a semi-arid shrubland in Argentina with a history of burns and can be considered as analogous to that of the seed bank, which justifies the use of the term bud bank (Klimešová & Klimeš 2007). However, further studies that address why these grasslands have such small seedbanks are needed: Has grassland management, including the use of fire, led to the selection of species that depend more on resprouting, but less on the soil seed bank? Is the small seedbank an intrinsic feature of this system? What is the role of the soil seed bank in vegetation dynamics over time? These questions still cannot be clearly answered for grasslands in our study region, nor for most other tropical and subtropical grassland regions.

CONCLUSIONS

The old-growth grasslands in the highland region of the southern part of Brazil's Atlantic Forest biome are under threat of biodiversity loss due to the expansion of afforestation. Our study indicated that – due to the low seed density and prevalence of sprouting species in these natural grasslands – recovery of the vegetation from the soil seed bank likely is not effective. Indeed, even in well-conserved grasslands, species likely depend much more on underground structures than on seeds for maintenance of their populations. The seed bank of the highland grasslands of southernmost Brazil studied here is much smaller than that reported for other subtropical grasslands, and likely seed banks play little or no part in the regeneration of vegetation after a disturbance. Based on our findings, we can expect that active restoration with seed introduction will be necessary for restoration of grassland sites after use of pine plantations, and research on this is urgently needed if biodiversity losses in the region are to be reduced.

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TABLE S1: List of species present in the established vegetation (six sites) and in the soil seed bank in natural grasslands and under pine plantations (three sites) in Cambará do Sul, RS, Brazil. Origin: N = native; E = exotic.

Species	Origin	Established vegetation	Grassland seed bank	Pine plant. seed bank
Acanthaceae				
Stenandrium dulce (Cav.) Nees	N	X		
Alstroemeriaceae	N	X		
Amaranthaceae				
Pfaffia tuberosa (Spreng.) Hicken	N	X		
Apiaceae				
Centella asiatica (L.) Urb.	E	X		
Eryngium eriophorum Cham. & Schltdl.	N	X		
Eryngium horridum Malme	N	X		
Eryngium zosterifolium H. Wolff	N	X		
Araliaceae				
Hydrocotyle exigua (Urb.) Malme	N	X	X	
Hydrocotyle ranunculoides L. f.	N		X	
Asteraceae				
Achyrocline satureioides (Lam.) DC.	N	X		
Acmella bellidioides (Sm.) R.K. Jansen	N	X		
Ageratum conizoides L.	N	X		
Asteraceae sp	N	X		
Baccharis coridifolia DC.	N	X		
Baccharis crispa Spreng.	N	X		
Baccharis pentodonta Malme	N	X		
Baccharis riograndensis Teodoro Luis & J.E. Vidal	N	X	X	
Baccharis sp.	N	X		
Baccharis subtropicalis G. Heiden	N	X		

Baccharis tridentata Gaudich.	N	X	
Baccharis uncinella DC.	N	X	
Chaptalia exscapa (Pers.) Baker	N	X	
Chaptalia integerrima (Vell.) Burkart	N	X	
Chaptalia piloselloides (Vahl) Baker	N	X	
Chaptalia runcinata Kunth	N	X	
Chevreulia acuminata Less.	N	X	
Chevreulia revoluta A.A. Schneid. & R. Trevis.	N	X	
Chrysolaena flexuosa (Sims) H. Rob.	N	X	
Elephantopus mollis Kunth	N	X	
Eupathorium sp.	N	X	
Eupatorium inulifolium Kunth	N	X	
Eupatorium nummularium Hook. & Arn.	N	X	
Gamochaeta americana (Mill.) Wedd.	N	X	
Gamochaeta coarctata (Willd.) Kerguélen	N	X	X
Gamochaeta pensylvanica (Willd.) Cabrera	N	X	X
Gamochaeta simplicicaulis (Willd. ex Spreng.) Cabrera	N	X	
Hypochaeris catharinensis Cabrera	N	X	
Hypochaeris lutea (Vell.) Britton	N	X	
Lessingianthus sellowii (Less.) H. Rob.	N	X	
Noticastrum decumbens (Baker) Cuatrec.	N	X	
Perezia squarrosa (Vahl) Less.	N	X	
Senecio brasiliensis (Spreng.) Less.	N	X	

Stenocephalum megapotamicum (Spreng.) Sch. Bip.	N	X		
Stevia lundiana DC.	N	X		
Trichocline catarinenses Cabrera	N	X		
Vernonia	N	X		
Boraginaceae				
Moritzia dasiantha Fresen.	N	X		
Bryophyta				
Bryophyta	N	X		
Campanulaceae				
Lobelia camporum Pohl	N	X		
Lobelia hederacea Cham.	N		X	
Lobelia nummularioides Cham.	N	X		
Wahlembergia linarioides (Lam.) A. DC.	N	X	X	
Caryophyllaceae				
Paronychia chilensis DC.	E	X	X	X
Convolvulaceae				
Dichondra macrocalyx Meisn.	N	X	X	
Dichondra sericea Sw.	N	X	X	X
Cyperaceae				
Bulbostylis brevifolia Palla	N	X	X	X
Bulbostylis hirtella (Schrad. ex Schult.) Nees ex Urb.	N		X	X
Bulbostylis rugosa M.G. López	N		X	
Bulbostylis scabra (J. Presl & C. Presl) C.B. Clarke	N		X	
Bulbostylis sp.1	N	X		
Bulbostylis sp.2	N		X	
Bulbostylis sphaerocephala (Boeckeler) Lindm.	N	X	X	
Bulbostylis subtilis M.G. López	N		X	X
Carex phalaroides Kunth	N	X		
Cyperaceae	N	X	X	X

Cyperus aggregatus (Willd.) Endl.	N		X	
Cyperus hemaphroditus (Jacq.) Standl.	N		X	
Cyperus reflexus Vahl	N		X	
Cyperus rigens J. Presl & C. Presl	N		X	
Cyperus sp.	N		X	
Kyllinga odorata Vahl	N	X	X	X
kyllinga vaginata Lam.	N		X	X
Rhynchospora barrosiana Guagl.	N	X		
Rhynchospora brasiliensis Boeckeler	N		X	
Rhynchospora edwalliana Boeckeler	N	X	X	
Rhynchospora globosa (Kunth) C. Presl	N	X		
Rhynchospora marisculus Lindl. ex Nees	N	X		
Rhynchospora sp.	N	X		
Scleria distans Poir.	N	X		
Scleria sellowiana Kunth	N	X		
Ericaceae				
Agarista nummularia (Cham. & Schltdl.) G. Don	N	X		
Gaylussacia angustifolia Cham.	N	X		
Eriocaulaceae				
Paepalanthus catharineae Ruhland	N	X		
Euphorbiaceae				
Euphorbia peperomioides Boiss.	N	X		
Fabaceae				
Arachis burkartii Handro	N	X		
Lupinus sp.	N	X		
Trifolium riograndense Burkart	N	X		
Hypericaceae				
Hypericum brasiliense Choisy	N	X		

Hypericum cordatum (Vell.) N. Robson	N	X		
Hypoxidaceae				
Hypoxis decumbens L.	N	X	X	
Iridaceae				
Sisyrinchium micranthum Cav.	N	X		
Sisyrinchium palmifolium L.	N	X		
Sisyrinchium vaginatum Spreng.	N	X		
Linaceae				
Linum erigeroides A. St.-Hil.	N	X		
Malvaceae				
Byttneria pedersenii Cristóbal	N	X		
Melastomataceae				
Rhynchanthera brachyrhyncha Cham.	N	X		
Tibouchina gracilis (Bonpl.) Cogn.	N	X		
Orchidaceae				
Habenaria parviflora Lindl.	N	X		
Orobanchaceae				
Buchnera longifólia Kunth	N	X		
Oxalidaceae				
Oxalis articulata Savigny	N	X		
Oxalis bipartita A. St.-Hil.	N	X		
Oxalis brasiliensis Lodd., G. Lodd. & W. Lodd. ex Hildebr.	N	X	X	
Oxalis conorriza Jacq.	N		X	
Oxalis sp. 1	N	X		
Oxalis sp. 2	N	X		
Plantaginaceae				
Mecardonia procumbens (Mill.) Small	N	X		
Mecardonia tenella (Cham. & Schltdl.) Pennell	N	X		
Plantago australis sub. australis	N	X		X

Plantago australis sub. hirtella (Kunth) Rahn	N	X		
Poaceae				
Andropogon lateralis Nees	N	X		
Andropogon macrothrix Trin.	N	X		
Aristida flaccida Trin. & Rupr.	N	X		
Aristida laevis (Nees) Kunth	N	X		
Aristida venustula (Nees) Kunth	N	X		
Axonopus affinis Chase	N	X	X	
Axonopus cf. compressus	N	X		
Axonopus ramboi G.A. Black	N	X		
Axonopu pellitus (Nees ex Trin.) Hitchc. & Chase	N	X	X	
Axonopus suffultus (J.C. Mikan ex Trin.) Parodi	N	X		
Chascolytrum poomorphum (J. Presl) L. Essi, Longhi-Wagner & Souza-Chies	N	X		
Chascolytrum subaristatum (Lam.) Desv.	N	X		
Chacolytrum uniolae L. Essi, Longhi-Wagner & Souza-Chies	N	X		
Chascolytrum lamarckianum L. Essi, Longhi-Wagner & Souza-Chies	N	X		
Danthonia montana Döll	N	X		
Danthonia secundiflora J. Presl	N	X		
Dichanthelium saburolum (Lam.) Gould & C.A. Clark	N	X	X	X
Digitaria violascens Link	S		X	X
Eragrostis lugens Nees	N	X	X	
Eragrostis polytricha Nees	N	X		
Gymnopogon sp.	N	X	X	
Paspalum lepton Schult.	N	X		

Paspalum maculosum Trin.	N	X	
Paspalum notatum Flüggé	N	X	X
Paspalum pauciciliatum (Parodi) Herter	N	X	
Paspalum plicatulum Michx.	N	X	
Paspalum polyphyllum Nees ex Trin.	N	X	
Paspalum pumilum Nees	N	X	X
Piptochaetium montevidensis (Spreng.) Parodi	N	X	
Poaceae	N		X
Poaceae sp 1		X	
Schizachyrium tenerum Nees	N	X	X
Setaria vaginata Spreng.	N	X	
Sorghastrum scaberrimum (Nees) Herter	N	X	
Sporobolus camporum Swallen	N	X	
Steinchisma hians (Elliott) Nash	N	X	
Nassella vallsii (A. Zanin & Longhi-Wagner) Peñail.	N	X	
Polygalaceae			
Polygala australis A.W. Benn.	N	X	
Polygala brasiliensis L.	N	X	
Polygala campestres Gardner	N	X	
Polygala pumila	N	X	
Polygala linoides Poir.	N	X	
Polygala pulchella A. St.-Hil. & Moq.	N	X	
Polygala sabulosa A.W. Benn.	N	X	
Polygala sp. 1	N	X	
Polygala sp. 2	N	X	
Primulaceae			
Anagallis mínima (L.) E.H.L. Krause	N	X	X

Rubiaceae				
Borreria capitata (Ruiz & Pav.) DC.	N	X	X	
Borreria tenella (Kunth) Cham. & Schltdl.	N	X		
Diodia radula (Willd.) Cham. & Schltdl.	N	X		
Galium humile Cham. & Schltdl.	N	X	X	X
Galium richardianum (Gillies ex Hook. & Arn.) Endl. ex Walp.	N		X	
Oldenlandia salzmannii (DC.) Benth. & Hook. f. ex B.D. Jacks.	N	X		
Richardia humistrata (Cham. & Schltdl.) Steud.	N	X		
Selaginellaceae				
Selaginella sp.	N	X		
Sphagnaceae				
Sphagnum sp.	N	X		
Verbenaceae				
Glandularia sp.	N	X		
Verbena hirta Spreng.	N	X		
Verbena montevidensis Spreng.	N	X		
Ni				
Sp 01	N	X		
Sp. 02	N	X		
Sp. 03		X		
Sp. 04		X		
Sp. 05		X		
Sp. 06	N	X		
Sp. 07	N	X		
Sp. 08		X		
Sp. 09		X		
Sp 10		X		
Sp 11			X	

TABLE S2: Results of analysis of variance (randomization test) of species richness, seed density and composition of natural grasslands (n=6) and pine plantation sites (n=3) in the highland grasslands in Cambará do Sul, Rio Grande do Sul state.

	Mean Value (SD)	*SS*	*P*-value
Richness		15.68	0.202
Grassland Seed Bank	4,4 (3,71)		
Pine Plantation Seed Bank	1,6 (0,8)		
Density		5.04E+06	0.259
Grassland Seed Bank	2487 (2246)		
Pine Plantation Seed Bank	900 (561)		
Composition		1.7564	**0.002**

CAPÍTULO 2

Restoring subtropical grasslands degraded by Pine plantations: testing methods to overcome biotic and abiotic filters

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Restoring subtropical grasslands degraded by Pine plantations: testing methods to overcome biotic and abiotic filters

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Abstract

Tree planting is among the main causes of land conversion in developing countries. Often established in formerly non-forest ecosystems, the so-called afforestation, generates many changes in local environmental conditions, with negative impacts on biodiversity, ecosystem services, and the herbaceous layer community. Highland grasslands of southern Brazil are rich in species and have a high level of endemism, however, they have also been suffering from afforestation. Some of these converted areas currently have a legal obligation to be restored however there is no experience of restoration of this type of degradation subtropical grasslands. In our study we aimed to test: (1) different ways of removing the remaining pine litter after clearcutting and its influence on the vegetation recovery and (2) if hay transfer is efficient for introduce species from reference area. Our results showed that pine litter removal is fundamental to increase vegetation cover and species richness and hay introduction had positive results species richness increase and only for summer hay. However, the biological types that make up the treatments did not approach the reference area two and a half years after the start of the experiment. Our data indicates that first colonizers, sedges and rushes remaining from the seed bank post-silviculture may cause a priority effect on the development of the restored community.

Keywords: seed bank, priority effect, pine litter, hay transfer, afforestation

Implications for Practice

- After use for pine plantation, litter removal is fundamental to promote grassland vegetation recovery and target species introduction
- Litter removal by burning shows be not effective in the highland grasslands of southernmost Brazil
- Actions to control the remaining seed bank should be considered to prevent the degraded area to follow a different course from that of the reference area
- Summer hay transfer increases species richness but not target species

INTRODUCTION

The conversion of natural ecosystems into areas with intensive soil use continues to grow expressively and tree plantations are among the most rapidly increasing land uses types, principally in developing countries (Zhang, Zhang, Yang, Wu, & Huang, 2014). Often established in formerly non-forest ecosystems, the so-called afforestation (practice of planting trees where they did not occur historically) generates many changes in local environmental conditions due to shading, increased humidity and litter accumulation, with negative impacts, often for long periods of time, on biodiversity, ecosystem services, and the herbaceous community (Berthrong et al. 2009; Veldman et al. 2015b). Some of these changes have impacts for long periods of time and leave legacies that remain even when areas are not used anymore as tree plantations (Koch et al., 2016; Leidinger et al., 2017; Torchelsen et al., 2018).

In southeastern South America, the expansion of silviculture started in the late 1980s (Gautreau & Vélez, 2011) and continues to be encouraged by public policies (Vega, Baldi, Jobbágy, & Paruelo, 2009). In the highland grasslands of southern Brazil, *Pinus elliottii* and *Pinus taeda* are the mainly species used in afforestation. Hermann et al. 2016 show a decrease in grassland cover of 17% only in 6 years for part of the highland grasslands of southern Brazil and confirm afforestation of former grassland areas as the main driver. These highland grasslands of southernmost Brazil are located at the southern tip of the Atlantic Forest Biome and are known for their diversity (Andrade, Bonilha, Ferreira, Boldrini, & Overbeck, 2016), high endemism levels (with 25% of their flora endemic) (Iganci et al., 2011) and cultural landscape, associated with the figure of the gaucho and extensive livestock production.

However, currently many of these tree plantations, established in originally grasslands areas, have been or are being due to enforcement of environmental legislation or economic reasons, cut and not be used for another tree cycle. As the current consequence, after clear cutting, when inside conservation units, these areas are left for passive regeneration without any type of restoration action, and usually shrub-dominated systems develop, often with considerable abundance of pine trees that are invasive in the region (Koch et al., 2016). When located in private areas but at the buffer zone these areas have restricted use, being one of the low impacts uses permitted in legislation the extensive livestock, which allows the recovery of several ecosystem services.

Although there is restoration demand, experiences in the restoration of non-forest ecosystems still are at the beginning in Brazil and South America in general (Gerhard E Overbeck et al., 2013), and also for the specific case grasslands degraded by use of Pine plantations. Appropriate restoration techniques still need to be developed. Conceptually, restoration aims to remove or manipulate assembly filters generated by environmental degradation (Hulvey & Aigner, 2014; Török, Helm, Kiehl, Buisson, & Valkó, 2018). Usually, both abiotic filters (for example, changed substrate conditions) and biotic filters (changed plant community composition) need to be addressed in restoration, as both components of the environment have been affected by degradation (Andrade et al., 2015). In the case of freshly cut pine plantations, the removal of accumulated litter (abiotic filter) to substrate prepare, helping in the conditions of moisture and light for the establishment of desired species such as in the reference area, and species introduction are fundamental for overcome environmental barriers (Navarro-Cano, Barberá, & Castillo, 2010) and achieve post-silvicultural restoration objectives. To overcome biotic filters, several techniques of plant introduction exist and are principally applied on the northern hemisphere (Kiehl, 2010). One method which presents good results for species introduction in North hemisphere is hay transfer (Baasch et al., 2012; Coiffait-Gombault et al., 2011; Kiehl, 2010; Kiehl & Wagner, 2006). However, few experiences exist for neotropical grasslands (Le Stradic, Buisson, & Fernandes, 2014) and subtropical grasslands have only one experience until now (Thomas et al, 2018) but with another history of degradation.

Considering the need for restoration of former pine plantation site and the scarcity of experiences with grassland restoration in southern Brazil, our study aims to evaluate the possibility to overcome barriers imposed by afforestation and to promote reestablishment of target species. We have three questions for understand which actions after the cutting of the trees are important to promote the recovery of the graasland converted area. (1) Is the removal of litter necessary for the restoration of vegetation? (2) What is the most effective treatment for the removal of pine needles for vegetation recovery? (3) Is possible introduce species of reference area by hay transfer? And exists a more promising season for species introduction by hay?

METHODS

Study area

The study was conducted close to Serra Geral National Park, in the highland region of Rio Grande do Sul state, southern Brazil (29°04'14'' S and 50°00' 36'' W). Altitude is about 1,000 m, and regional climate is Cfb according to Köppen climate classification, with mean annual precipitation of 1,881 mm distributed evenly throughout the year. Original vegetation of the region is formed by mosaics of species-rich natural grasslands, dominated principally by caespitose grasses such as *Andropogon lateralis* Nees and *Sorghastrum pellitum* (Hack.) Parodi, Araucaria Forest, and Nebular forest (Leite & Klein 1990). Grasslands are being used for extensive livestock production, with additional use of fire as a management tool, forming a cultural landscape. In the past 20 years public policies have stimulated forestry expansion (Gautreau & Vélez 2011; Hermann et al. 2016) over grasslands. Some of these plantings had been established in areas where they are illegal, such as in Permanent Protection Areas, or in the buffer zone of Conservation Units. This has led to a liability of many hectares of degraded areas where restoration of original vegetation is obligatory.

The experiment was implemented in a private area, with 280 ha of Pinus cultivation for 30 years, located in the buffer zone of the National Park. Tree cutting started at the site in January 2015. After clear cutting, a layer of pine twigs and pine needles with a depth of about 10 cm remained in the study area. In March 2015, the experiment was installed Fig. 1.

(A) (B)

Figure 1: Experimental area. (A) post shallow cut area with branches and litter. (B) the area in preparation for the installation of the experiment.

Experimental design

The experiment had a bi-factorial design, with a combination of 16 different treatments aiming to improve substrate conditions by removal of Pine litter and seed introduction. The substrate levels (Factor 1) had the objective of removing the thick layer of needles and prepare the soil at the experiment site, with four levels: Control (C) - no removal action; Removal (R) – manual removal of the whole litter layer until reaching the intact soil surface; Scarification (S) - removal of entire litter layer and scarification of the topsoil; Burn (B): litter removal by burning. The hay introduction (Factor 2) were designed to evaluate the potential role of seed input via hay transfer at different seasons, also with four levels: Control (1) - no hay introduction; Summer hay (2) - hay collected and introduced at the end of the summer; Spring hay (3) - hay collected and introduced at the end of the spring; Summer and spring hay (4): application of hay in both seasons.

Treatments were established in experimental plots of 9 m^2, with distance of 1 m between plots, forming a total of 16 treatments plots per block. We had a total of five blocks (used as repetition) with randomly assigned levels. Inside each experimental plot, four sampling units of $1m^2$ (situated 50 cm from the edge) were allocated to monitor vegetation regeneration. Vegetation was monitored over two and a half years. In the removal level, pine litter and twigs were removed manually with rakes and showels. In the scarification level, we additionally disturbed the upper soil layer approximately 5 cm. Burns were set by the help of a drip torch fuel with gasoline

Reference area and hay collection

Data about species composition in reference areas were collected in December 2014 at six grassland sites in the region, with 10 plots of $1m^2$ randomly allocated per site. Cover of all vascular species was registered using the Londo decimal scale (Londo 1976). In one of the reference areas, the vegetation was cut with a grass trimmer in March 2015 (i.e. southern hemisphere summer) for the summer hay level and in November 2015 for the spring hay level. At each cutting date, hay was left to dry at air temperature for three days before weighing and transposition. Hay was homogenized and spread, at each of the dates, in the experimental plots assigned for the treatments, using ca. $500g/m^2$. Thus, the level with summer and spring received this quantity of hay twice.

Vegetation monitoring

Over two and a half years, we carried out four vegetation surveys to monitor plant recolonization in each study unity (permanent plot); here, we use data from 2015 and 2017. We recorded in 2015, in four sampling units of $1m^2$ and, in 2017 in two sampling units total vegetation cover, cover of open soil and cover of pine litter, as well as individual cover of all species, using the Londo scale (Londo 1976). For all treatment combinations, we presented mean values per $1m^2$. Species were classified according to their biological type (bryophytes, graminoids[1], sedges and rushes, herbs and shrubs) as well as into target (species that occurred in the reference areas) or non-target species (without occurrence in the reference area).

Data Analysis

Mean vegetation cover, total species richness, target species richness and cover per biological type were analyzed, separately for the two sampling dates, by randomization test implemented in Multiv Software (Pillar 2014) using euclidean distance as resemblance measure with 10,000 iterations. To evaluate differences in

[1] Graminoids included all monocot species except for Cyperaceae and Juncaceae. Species from the latter two families were considered in a separate group – sedges and rushes – due to the ruderal character of these species, as evidenced e.g. in their importance in the soil seed bank in the study region (Vieira & Overbeck, submitted).

composition among treatments, we performed a randomization test based on a chord distance as resemblance measure and 10,000 iterations (Pillar & Orlóci, 1996). All *p*-values generated by randomization were adjusted by Bonferroni correction. The ordination diagram by Principal Coordinates Analysis (PCoA), using chord distance as resemblance measure, was conducted to explore general patterns based on cover data of experimental plots of the year 2017. We used indicator species analysis with labdsv package (Roberts 2016), for each factor, to identify species associated to the experimental treatments, using the in R software (R Development Core Team 2017).

RESULTS

Species richness and composition of reference areas

A total of 168 species distributed in 31 botanical families were recorded in the reference area (Table S4). Of these, only two were exotic but naturalized in the region (*Centella asiatica and Paronychia chilensis*), which indicates a good conservation status. The mean richness recorded was 22 species/m². *Andropogon lateralis* (21%; mean value across sites*)*, *Sorghastrum scaberrimum* (12.8%), *Axonopus pellitus* (7.5%) and *Axonopus affinis* (5%) are the species with larger cover means. Considering the composition from biological types point view, graminoids were the group with predominant coverage per square meter (45%), followed by herbs (15%), shrubs (8%) and finally sedges and rushes (7%). Due to the objective of reintroducing species present in the reference area in degraded areas, all species recorded in the reference grasslands were classified as target species.

Substrate removal level efficiency

Nine months after implementation of the experiment, the control level for substract factor shows mean values as: vegetation cover = 7,3%; bare soil = 1,4%; litter = 95% and rocks = 0%.an efficiency for removal and scarification treatments

Burn level did not show the expected efficiency for litter removal even with the application of fuel. The litter caught fire only in the most superficial layer, approximately 2-3 cm, and mostly did not affect the entire plots. Likely the high humidity of the region impeded development of more effective burns.

Vegetation cover in experimental plots

In levels with effective removal of litter, i.e. the manual removal and the manual scarification levels, vegetation cover increased soon after the start of the experiment (Fig. 2). Two years after implementation of the experiment, the substrate levels (factor 1) led to significant differences between control in vegetation cover (p = 0.0007), species richness (p = 0.0007), target specie richness and target specie cover (p = 0.049 and p = 0.016, respectively; see Table 2 and Table S2 for complete results). Vegetation cover was significantly higher in scarification (70%) and removal (62,2%) levels, compared to burn and control (Table 2). The removal level showed significative difference for all analyzed parameters in comparison to control, followed by scarification level, which was efficient only for increase vegetation cover and species richness different. Burn level did not show significant differences from control for any of the observed parameters.

Two years after the start of the experiment, the hay levels (factor 2) led to significant differences only for species richness and only between the control (8.6 species) and the summer hay level (12.6 species; p = 0.035); the other levels had intermediate values that did not differ from the other levels. The interaction between factors (f1 x f2) was not significant for any of the observed parameters.

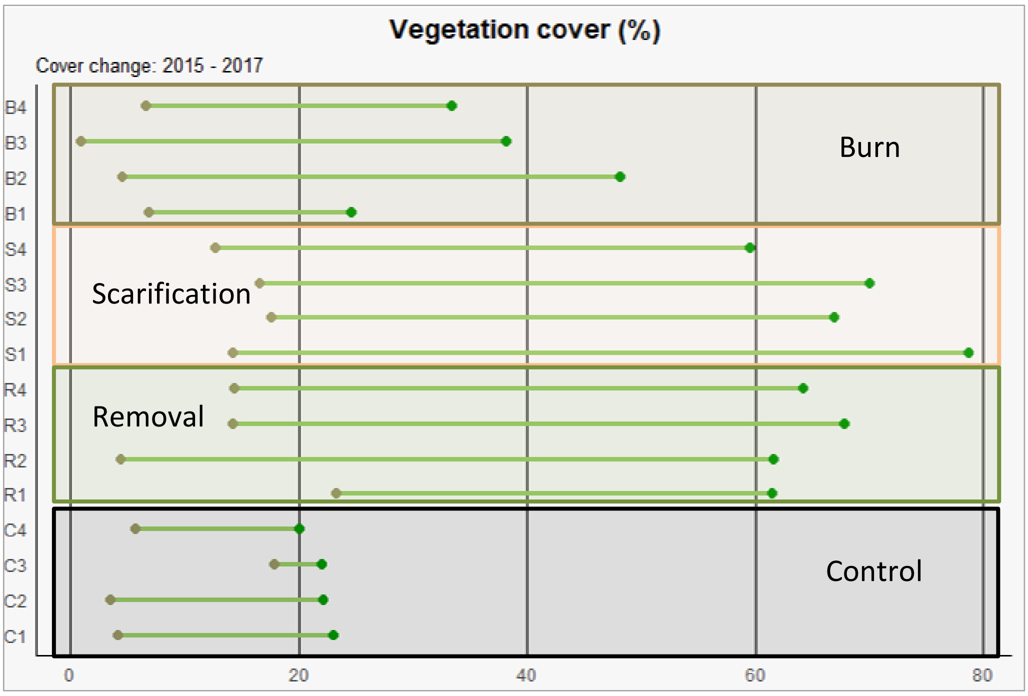

Figure 2. Dumbbell plot of vegetation cover means of each treatment 33 months after the start of experiment. Letters represent substrate levels (C. Control, R. Removal, S.

Scarification, B. Burn) and numbers represent hay levels (1. Control, 2. Sommer hay, 3. Spring hay, 4. Sommer and spring hay. Note that the first sampling was conducted 9 months after implementation of treatments, explaining the differences at the first sampling date.

Table 2. Values of vegetation cover, species richness and target species in 2017 per square meter according to each factor and between factors. *p* values observed after Bonferroni corrections = not significant (p> 0.05); **p< 0.01 and *** p<0.001.

		Vegetation cover	Species richness	Target species richness	Target species cover
Substrate level (f1)		***	***	*	*
	Control	21.77 ^{b}	7 ^{b}	5.2 ^{b}	18.53 ^{b}
	Removal	62.42 ^{a}	12 ^{ac}	8.7 ^{a}	37.65 ^{a}
	Scarification	70.02 ^{a}	12 ^{ac}	9.4 ^{ab}	39.03 ^{ab}
	Burn	36.8 ^{b}	10 ^{bc}	7.2 ^{ab}	23.12 ^{ab}
Hay level (f2)		ns	*	ns	ns
	Control	50.2	8.6 ^{b}	6.5	27.8
	Summer	48.6	12.6 ^{a}	8.9	33.3
	Spring	49.7	9.4 ^{ab}	7.4	31.3
	Summer & Spring	42.5	11.6 ^{ab}	7.6	25.5
Interaction f1 x f2		ns	ns	ns	ns

Biological type and species composition

Over the course of the experiment, we recorded a total of 132 plant species in the experimental treatments. At the 2017 sampling date, 67 species were found and, 45 of these were target species. The species classification according to biological types showed that all treatments had a vegetation structure different from the reference area, and these differences increased with time (Fig. 3).

At the beginning of the experiment, in 2015, there was no significant difference of biological type cover among levels and graminoids were the group with highest proportion of cover (Fig. 3a). After two years, all substrate removal levels showed significantly higher cover of sedges and rushes than the control plots (*p* = 0.043). Cover of sedges and rushes exceeded that of graminoids after two years (Fig. 3b). The hay factor (f2) did not present significant differences for any of the biological types in 2015 and 2017 (Fig. 3d and 3e). The interaction between factors also did not differ statistically (for complete results, see Appendix S3).

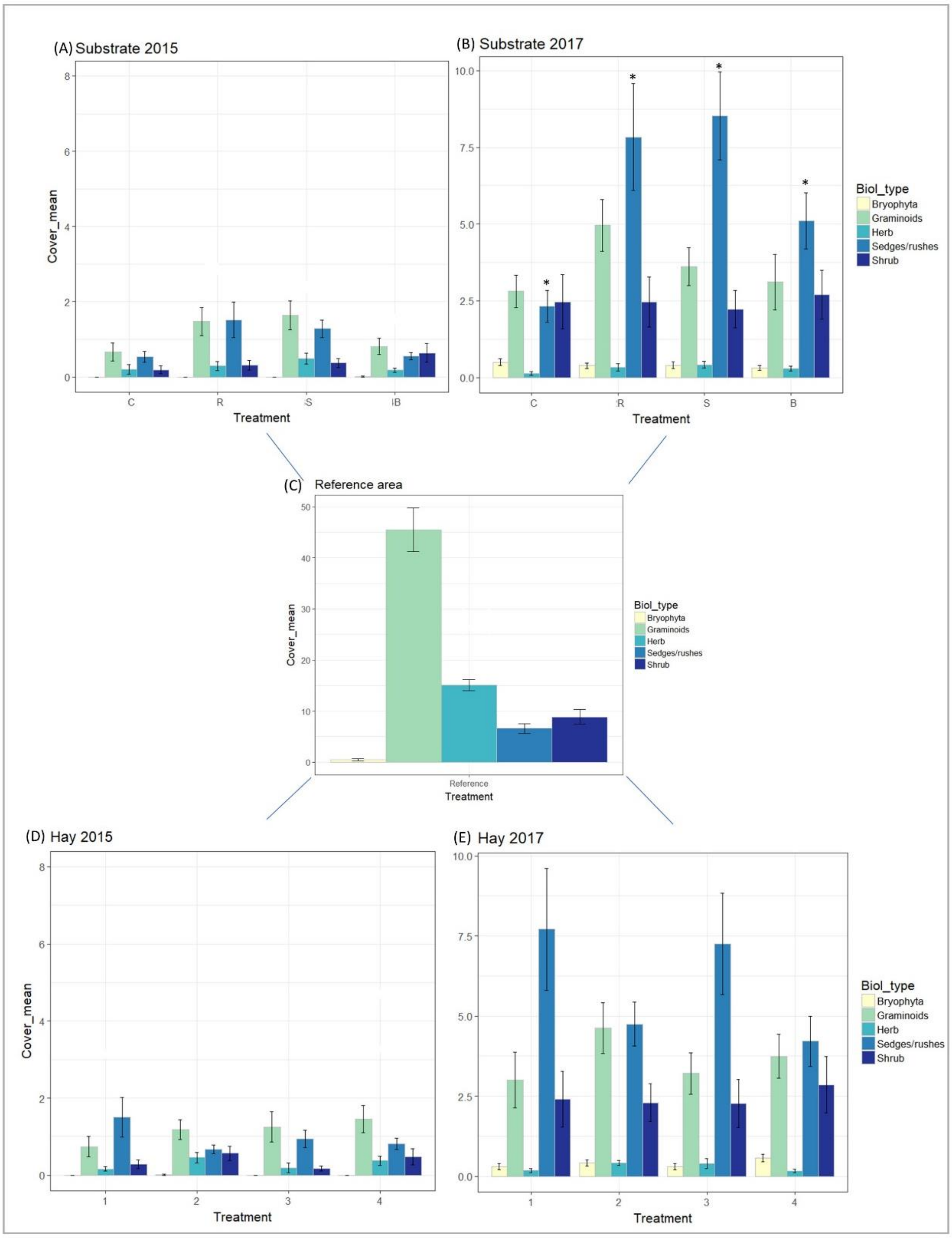

Figure 3. Percentation of vegetation cover (m^2) change over time per biological type per treatment. Substrate factor (f1) (C. Control, R. Removal, S. Scarification, B. Burn) and numbers represent hay factor (f2) (1. Control, 2. Summer hay, 3. Spring hay, 4. Summer and spring hay. Please note the difference in scale on the y-axis between the figures. Asterisk indicates statistical significance among levels, for the sedges and rushes (Detailed results in Table S3).

In terms of species composition, the substrate factor led to significant differences among some levels (p = 0.009). Composition of control plots differed significantly from plots with litter removal and plots with scarification. Additionally, scarification plots differed from burn plots (which did not differ from the control). In contrast, the hay transfer factor did not lead to differences. The interaction between factors was also not significant (Appendix S2). In terms of composition for substrate factor (f1), the contribution of target and non-target species was similar among levels, except for the control level. The three targets species with greatest cover in the experimental plots were *Rhynchospora edwalliana, Dichanthelium sabulorum and Rhynchospora barrosiana,* with differences in proportions among levels. The most abundant species of the reference area, *Andropogon lateralis*, was recorded in all substrate levels groups except for the control, although with low cover values (Fig. 4). The non-target species with higher cover mean was a grass, *Agrostis montevidensis,* followed by *Rhynchospora brasilisensis* (Fig. 4). Although they were classified as non-target species because were not recorded in the reference area, all non-target species with the highest contribution recorded in experimental area were native species and have known occurrence in the region.

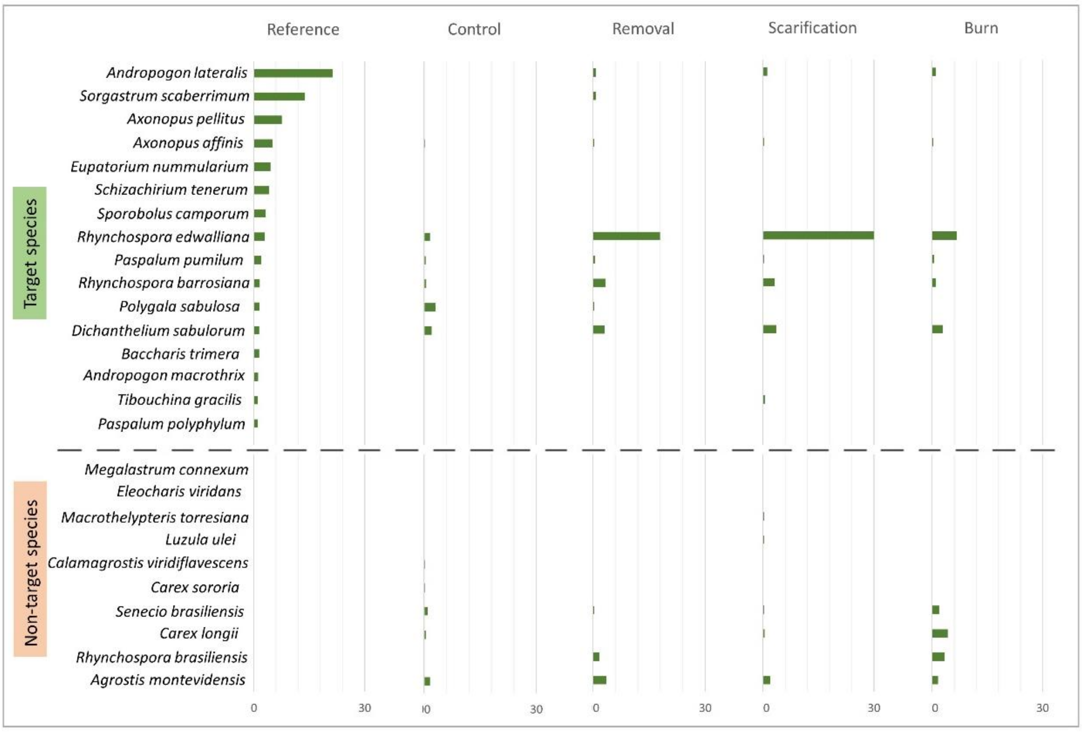

Figure 4. Species cover over mean/m² of target and non-target (factor 1) 33 months after the start of the experiment. Only species with cover mean more than 1% per square meter in the reference, and with ≤0.02 in the experimental area are shown.

The indicator species analysis revealed few species as indicative of the different levels combinations (treatment). (Table 2). For the substrate factor, only one Poaceae species, *Danthonia secundiflora,* one of the target species, was selected as indicator species (for the removal level). Two Cyperaceae and one Asteraceae where indicators for the scarification level, and one for the burn level. The hay factor only had species significantly related with the levels summer hay (2) and summer and spring hay (4). Of these, one target species *Chascolytrum subaristatum* was indicator for summer hay level.

Table 2. Results of Indicator species per factor, showing species significantly associated to different levels of each factor. Target species are indicated with an asterisk.

	level	Species	Indval	*p*-value
Substract factor (f1)	Removal	**Danthonia secundiflora*	0.379837	0.023
	Scarification	**Bulbostylis sphaerocephala*	0.578146	0.001
	Scarification	*Rhynchospora edwalliana*	0.537175	0.002
	Scarification	**Grazielia nummularia*	0.317271	0.009
	Burn	*Carex longii*	0.380121	0.014
Hay factor (f2)	Summer hay	*Agrostis montevidensis*	0.411299	0.003
	Summer hay	**Chascolytrum subaristatum*	0.243678	0.038
	Summer and Spring hay	*Carex sororia*	0.208333	0.018

DISCUSSION

Two and a half years after the beginning of the experiment our results show that the restoration treatments are distant in terms of the composition of the reference area although most of the species found in the treatments are target species although with a considerable advance when compared with control treatment. The dominant biological type group of the reference area was replaced by sedges and rushes on the restoration treatments, indicating a change in the community structure.

Substrate levels

Our study indicates that the litter layer act as abiotic filter for plant recovery, limiting the establishment of new species and preventing the germination of species present in the seed bank. Our results clearly show that litter removal was important to overcome this environmental barrier for all analyzed parameters (vegetation cover, species richness, target species richness and target species cover). Similar results about the negative litter impact on germination, establishment and richness in grasslands were found by Xiong & Nilsson (1999) in a meta-analysis by Loydi et al. (2014) in grassland in Central Europe, where litter reduced grassland vegetation cover and species richness. It has even been shown that fungi which have symbiotic relationship with pine trees have reduced ectomycorrhizal development at sites with dense layers of pine litter due to drastic local changes generate (Baar & Kuyper, 1998).

In our study, levels that presented better response for the increase of vegetation cover were the removal and scarification levels, which were the levels in which there was effective removal of all litter, leaving the soil exposed. The burn level did not show significant differences in comparison to the control treatment; this treatment did not prove to be an effective alternative for needles removal in a humid region as the Campos de Cima da Serra. The moisture retained in the litter layer and/or the rather dense packing likely prevented the effective burning of the needles. However, future research should address possible differences between seasons or the impact of litter deposition overtime on vegetation recovery; flammability of pine litter in the region has not yet been addressed in specific studies (e.g. Varner et al. 2015).

Litter removal, with or without scarification of the soil surface, proved successful in increasing vegetation development. While we did not evaluate the physical effects of these levels, other studies also showed that the manipulation of this abiotic barrier is important to promote increased light input and reduce local humidity (Loydi et al., 2014), promoting more similar physical conditions to a natural grassland. Additionally, accumulated litter act as a seed trap, retaining seeds and impeding seed arrival on the soil where they can germinate. Only few seeds with a favorable shape can overcome this layer and reach the soil, which causes a selection of species with attributes that allow their establishment in the local (Bueno & Baruch, 2011; Ruprecht & Szabó, 2012). Sedges and rushes are known by the high seed production and seed persistence in the soil (Marco & Páez 2000; Maia et al. 2003; Allessio Leck & Schütz 2005) in consequence, these attributes favor their colonization and rapid establishment in a place where there is no competitiveness due to the absence or low contribution of other biological types. This effect can be seen over time in our study area, and even stronger in the removal and scarification levels than in the control, where vegetation development continued to be slow.

Removal and scarification levels showed, at the beginning of the experiment, grasses as the biological type with the highest contributions of cover for these levels. However, the removal of the physical barrier that covered the ground and the additional scarification apparently activated germination from the soil seed bank, which after use as pine plantation is composed largely by sedges (Vieira & Overbeck, 2020) leading to the formation of a community that greatly differed to that of the reference area in terms of composition and structure (Fig. 2b and 2c). Our results suggest that the initial colonization by perennial species as *Rhynchospora edwalliana*, *Rhynchospora barrosiana* and *Bulbostylis sphaerocephala,* which have high seed production, can, generate a priority effect in the of the new community composition which can be propagated over time (Fukami, 2015; Hobbs et al., 2006; Körner, Stöcklin, Reuther-thiébaud, Pelaez-riedl, & Körner, 2008), as indicated by the results of the 2017 survey.

Priority effects can be classified in two types of mechanisms: niche preemption and niche modification. In the first one, niche preemption, changes alter species identity, but do not consider functional groups, guilds, or biological types. Under the niche modification mechanism, the changes caused by the first colonizes can cross functional groups, guilds or biological types (Fukami, 2015). In our study area, the composition in substrate levels at the end of the experiment shows changes in biological type structure, indicating a niche modification the levels.

Hay levels

At first glance, hay transfer in our study only had a limited contribution to overcome seed limitation at the experimental site: Hay transfer had a significant effect only for species richness, but not species cover, and only for hay collected in summer. With *Chascolytrum subaristatum*, a target species with high frequency in the reference grassland was among the indicator species for the summer hay level. In contrast, the few available studies that tested hay transfer in tropical and subtropical grasslands e. g. by Le Stradic et al. (2014) in rupestrian grasslands degraded by quarrying, and by Thomas et al. (2018) and Pilon, Buisson, & Durigan (2018) in subtropical grassland and tropical savanna, respectively, degraded by invasive grasses, did not evidence any effects for hay treatments. One possible cause for this may be that these grasslands are largely composed of long-lived species that effective mechanisms for resprouting after biomass loss e.g. by fire and grazing, while they do depend less on reproduction by seed (e.g. Veldman et al. 2015). Our study thus is a first indication that even in these grasslands, effects do exist, even though apparently not as pronounced as in temperate grasslands; interestingly, the selected indicator species present in the target community (*C. subaristatum*) was a C3 grass, i.e. a temperate element of the grassland. However, apart from this contribution to overcome seed limitation, we perceive that hay introduction had important effects on vegetation recovery. Levels that did not receive hay (control) or remained exposed for seven months until hay application (spring hay), had proportionally higher cover of sedges and rushes, as showed by Fig. 2d and 2e. In contrast, levels that received summer hay, i.e. were subject to hay transfer soon after installation of the experiment, showed lower sedges and rushes cover mean. We thus can infer that the thin layer of hay acted to reduce sedges and rushes germination. These findings indicate that hay avoid or reduced sedges and rushes priority effects, however, these were appearing as the hay was decaying. Török et al. (2011) found a similar hay effect in grasslands recovery on former croplands, where the main effect of hay introduction was to prevent the growth of weeds.

The introduction of species by hay was not enough to increase the parameters of target species richness and target species cover. It is known that the highland grasslands of the Atlantic Forest biome of southern Brazil are historically under fire and grazing disturbances (Behling & Pillar, 2007) and these disturbances conditions selected principally a species composition formed mostly by resprouters species from above and belowground buds (Fidelis, Appezzato-da-Glória, Pillar, & Pfadenhauer, 2014; Gerhard Ernst Overbeck & Pfadenhauer, 2007) rather than seeders species.

Vegetation development relative to the reference grasslands

Our results indicate that, even two and a half years after implementation of the experiment, plots of majority treatments are at initial state of recovery, with low richness of target. This result has implications, on the one hand, regarding interpretation of the degradation state and the recovery trajectory of the vegetation, and on the other hand for future management activities and monitoring. Possibly, other interventions may be necessary that aim to manage vegetation cover in a way that desired (target) species are benefitted, while less-desired species are reduced or eventually excluded from the system (see e.g. Cole, Prober, Lunt, & Koen, 2016). Maybe the number of hay transposition events was not enough to overcome the propagules lack over time, and other events of hay introduction should be considered to have also an effect on maintaining the biotic legacy (sedges and rushes seed bank) left by pine planting, in addition to richness increasing. This also means that it will be necessary to develop different sets of indicators that should be used in different restoration phases: at the beginning of the experiments, indicators based on diversity of the reference systems may not be useful, in contrast to later phases (see also Rocha-Nicoleite, Campos, Colombo, Overbeck, & Müller, 2018) who discuss the need of adaptive indicator systems for forest restoration after severe degradation).

CONCLUSIONS

Our study is the first to present results from restoration trials in the highland grasslands in subtropical southern Brazil and – despite a slowly growing body of literature – still one of the rather few studies on restoration of tropical and subtropical grasslands. Our aims were to test different ways to overcome abiotic and biotic barriers after use as pine plantations. The consequences of the absence of graminoid functional type on the experimental area influenced vegetation structure by the strategy of the new colonizers, taking the experimental area to different vegetational structure from the reference area.

Our data indicate that the pine litter layer is a severe barrier for vegetation recovery, but that removal of the litter triggers plant establishment from the soil seed bank that is mostly composed by sedges and rushes species. Hay transfer – that increased species richness for one of the two collection dates – had the additional effect of reducing this spontaneous establishment of sedges and rushes that may be negative for recovery of the plant community. The results with hay transfer indicate that further research on timing of seed collection, quantity of hay transferred and composition of the donor community is important, but that it also is necessary consider other methods of species introduction, e.g. direct seeding or planting. This might avoid practices such as exotic species sowing that aim only soil cover and can, and are, easily be used on a landscape scale. Overall, our results point that efforts to restore them will be time-demanding and possibly costly. Nonetheless, restoration is important for these grassland in a biodiversity hotspot that are highly threatened by land use change if international conservation aims are to be met.

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Table S1. Scheme illustrating two factors and combinations of levels between them.

		Substrate levels (f1)			
		C (control)	R (removal)	S (scarification)	B (burn)
Hay levels (f2)	1 (control)	C1	R1	S1	B1
	2 (summer hay)	C2	R2	S2	B2
	3 (spring hay)	C3	R3	S3	B3
	4 (summer and spring hay)	C4	R4	S4	B4

Table S2. Results of permutation test for vegetation cover mean, richness, composition, target species richness and target species cover per factor and, among contrast between levels. Sum of squares (SS), significance (*p*-value). *P* values observed after Bonferroni corrections.

	Vegetation cover		Richness		Composition		Target species richness		Target species cover	
	SS	*p*- value	SS	*p*- value	SS	*p*- value	SS	*p*- value	SS	*p*- value
Substrate (f1)	30123	**0.0007**	369.24	**0.0007**	4.098	**0.0091**	210.1	**0.049**	63.578	**0.0161**
Control x Removal	16524	**0.0007**	225.62	**0.0028**	1.438	**0.0105**	122.5	**0.0035**	36.577	**0.0021**
Control x Scarification	23281	**0.0007**	308.03	**0.0049**	2.25	**0.0217**	180.63	ns	42.025	ns
Control x Burn	2257.5	ns	70.225	ns	0.63003	ns	42.025	ns	2.1391	ns
Removal x Scarification	577.6	ns	6.4	ns	0.4116	ns	5.625	ns	0.18906	ns
Removal x Burn	6566.4	**0.0021**	44.1	ns	1.3043	ns	21.025	ns	21.025	ns
Scarification x Burn	11039	**0.0007**	84.1	ns	2.1621	**0.0357**	48.4	ns	25.202	ns
Hay (f2)	757.23	ns	212.54	**0.035**	1.8595	ns	63.342	ns	7.2082	ns
Control x Summer hay	25.6	ns	164.03	**0.042**	0.50902	ns	61.609	ns	3.0827	ns
Control x Spring hay	1.8062	ns	7.225	ns	0.22488	ns	8.1	ns	1.2426	ns

Control x Summer & Spring hay	585.22	ns	93.025	ns	0.85608	ns	13.664	ns	0.49212	ns
Summer x Spring hay	13.806	ns	102.4	ns	0.67701	ns	24.687	ns	0.39382	ns
Summer x Summer & Spring hay	366.03	ns	10	ns	0.51001	ns	16.042	ns	5.9651	ns
Spring x Summer & Spring hay	522.01	ns	48.4	ns	0.95779	ns	0.78704	ns	3.2466	ns
Substrate (f1) x Hay (f2)	669.58	ns	125.61	ns	4.9725	ns	108.51	ns	31.535	ns

Table S3. Results of permutation test for cover of graminoids, herbs, sedges/rushes and shrub biological type per factor and among levels. Sum of squares (SS), significance (*p*-value). *P* values after Bonferroni corrections.

	2015							
	Graminoids		Herb		Sedges/rushes		Shrub	
	SS	*p*-value	SS	*p*-value	SS	*p*- value	SS	*p*-value
Substrate (f1)	6.3107	ns	0.47882	ns	15.229	ns	3.2453	ns
Control x Removal	1.9076	ns	0.054084	ns	11.217	ns	0.32852	ns
Control x Scarification	3.5897	ns	0.22313	ns	4.3973	ns	0.82656	ns
Control x Burn	5.6415	ns	0.031641	ns	0.052584	ns	3.0941	ns
Removal x Scarification	0.26366	ns	0.057507	ns	1.5681	ns	0.11289	ns
Removal x Burn	0.98807	ns	0.16846	ns	9.7339	ns	1.4063	ns
Scarification X Burn	0.23092	ns	0.42282	ns	3.4882	ns	0.72227	ns
Hay (f2)	17.278	ns	0.48106	ns	9.8011	ns	5.2223	ns
Control x Summer hay	8.0966	ns	0.34775	ns	8.7217	ns	2.1743	ns
Control x Spring hay	11.249	ns	0.15992	ns	4.391	ns	0.30625	ns
Control x Summer & Spring hay	0.12166	ns	0.37317	ns	5.2981	ns	1.0798	ns
Summer x Spring hay	0.30161	ns	0.034209	ns	0.69284	ns	4.1399	ns
Summer x Summer & Spring hay	6.0268	ns	0.0013091	ns	0.34258	ns	0.16297	ns
Spring x Summe & Spring hay	8.7729	ns	0.046717	ns	0.054436	ns	2.5134	ns
Substrate (f1) x Hay (f2)	21.489	ns	2.9991	ns	23.322	ns	7.5987	ns

	2017							
	Graminoids		Herb		Sedges/rushes		Shrub	
	SS	*p*-value	SS	*p*-value	SS	*p*- value	SS	*p*-value
Substrate (f1)	39.82	ns	0.79087	ns	494.04	**0.0434**	1.742	ns
Control x Removal	35.858	ns	0.49506	ns	344.78	**0.0049**	0.081753	ns
Control x Scarification	10.149	ns	0.66	ns	363.45	**0.0084**	0.63756	ns
Control x Burn	2.082	ns	0.35784	ns	76.367	**0.0385**	0.15521	ns
Removal x Scarification	7.8533	ns	0.011837	ns	0.24619	ns	1.1759	ns
Removal x Burn	20.659	ns	0.011111	ns	96.617	ns	0.011674	ns

Scarification X Burn	3.0376	ns	0.045884	ns	106.62	ns	1.4219	ns
Hay (f2)	21.432	ns	1.4783	ns	95.959	ns	7.0584	ns
Control x Summer hay	12.051	ns	0.54884	ns	52.406	ns	1.2183	ns
Control x Spring hay	0.078677	ns	0.82793	ns	1.1576	ns	0.21389	ns
Control x Summer & Spring hay	0.68328	ns	0.0038463	ns	57.751	ns	2.1301	ns
Summer x Spring hay	10.161	ns	0.032435	ns	37.825	ns	0.40409	ns
Summer x Summer & Spring hay	18.164	ns	0.63012	ns	0.29769	ns	6.5839	ns
Spring x Summer& Spring hay	1.2177	ns	0.92196	ns	42.737	ns	3.6711	ns
Substrate (f1) x Hay (f2)	151.02	ns	1.2521	ns	297.8	ns	90.722	ns

Table S4. Complete list of species recorded in the reference area and in restoration treatments.

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Achyrocline satureioides (Lam.) DC.	x	x			x		x	x	x
Acmella bellidioides (Sm.) R.K. Jansen	x								
Agarista nummularia (Cham. & Schltdl.) G. Don	x								
Ageratum conizoides L.	x								
Agrostis montevidensis Spreng. ex Nees		x	x	x	x	x	x	x	x
Anagallis mínima (L.) E.H.L. Krause	x								
Andropogon lateralis Nees	x	x	x	x	x		x	x	x
Andropogon macrothrix Trin.	x								
Arachis burkartii Handro	x								
Aristida flaccida Trin. & Rupr.	x								
Aristida laevis (Nees) Kunth	x								
Aristida venustula (Nees) Kunth	x								
Asteraceae sp.	x								

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Austroeupatorium inulifolium (Kunth) R.M.King & H.Rob.			x	x	x	x	x	x	x
Axonopu pellitus (Nees ex Trin.) Hitchc. & Chase	x								
Axonopus affinis Chase	x	x	x	x	x	x	x	x	x
Axonopus cf. compressus	x								
Axonopus ramboi G.A. Black	x								
Axonopus suffultus (J.C. Mikan ex Trin.) Parodi	x				x	x			
Baccharis apicifoliosa A.A.Schneid. & Boldrini		x	x	x	x	x	x	x	x
Baccharis coridifolia DC.	x								
Baccharis crispa Spreng.	x	x	x	x	x	x	x	x	x
Baccharis milleflora (Less.) DC.			x	x	x	x	x		x
Baccharis pentodonta Malme	x								
Baccharis riograndensis Teodoro Luis & J.E. Vidal	x	x	x		x	x	x	x	x
Baccharis sp.	x								
Baccharis sphagnophyla A.A.Schneid. & G.Heiden		x							x

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Baccharis subtropicalis G. Heiden	x								
Baccharis tridentata Gaudich.	x								
Baccharis uncinella DC.	x	x	x	x	x	x	x	x	x
Borreria capitata (Ruiz & Pav.) DC.	x								
Borreria tenella (Kunth) Cham. & Schltdl.	x								
Bryophyta	x	x	x	x	x	x	x	x	x
Buchnera longifolia Kunth	x								
Bulbostylis brevifolia Palla	x								
Bulbostylis sp.1	x								
Bulbostylis sphaerocephala (Boeckeler) Lindm.	x	x	x	x	x	x	x	x	x
Byttneria pedersenii Cristóbal	x								
Calamagrostis viridiflavescens (Poir.) Steud.		x		x	x		x		x
Carex longii Mack.		x	x	x	x	x	x	x	x
Carex phalaroides Kunth	x								
Carex sororia Kunth		x	x	x	x		x		x
Centella asiatica (L.) Urb.	x								
Cerastium commersonianum Ser.				x			x		

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Cerastium rivulare Cambess.				x			x		
Chaptalia exscapa (Pers.) Baker	x								
Chaptalia integerrima (Vell.) Burkart	x								
Chaptalia piloselloides (Vahl) Baker	x								
Chaptalia runcinata Kunth	x								
Chascolytrum lamarckianum L. Essi, Longhi-Wagner & Souza-Chies	x								
Chascolytrum poomorphum (J. Presl) L. Essi, Longhi-Wagner & Souza-Chies	x								
Chascolytrum subaristatum (Lam.) Desv.	x		x	x	x		x	x	x
Chascolytrum uniolae L. Essi, Longhi-Wagner & Souza-Chies	x	x	x	x	x		x	x	x
Chevreulia acuminata Less.	x								
Chevreulia revoluta A.A. Schneid. & R. Trevis.	x								
Chloris sp.			x				x		

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Chrysolaena flexuosa (Sims) H. Rob.	x								
Cliococca sellaginoides (Lam.) C. M. Rogers & Mild					x		x		
Cyperus distans L.		x	x				x		x
Cyperus sp.					x	x			
Danthonia secundiflora J. Presl	x	x	x	x	x	x	x	x	x
Dichanthelium saburolum (Lam.) Gould & C.A. Clark	x	x	x	x	x	x	x	x	x
Dichondra macrocalyx Meisn.	x								
Dichondra sericea Sw.	x								
Diodia radula (Willd.) Cham. & Schltdl.	x								
*Eleocharis viridans*Kük. ex Osten			x		x		x	x	x
Elephantopus mollis Kunth	x								
Eragrostis lugens Nees	x								
Eragrostis polytricha Nees	x								
Eryngium ebracteatum Lam.				x			x		
Eryngium eriophorum Cham. & Schltdl.	x								

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Eryngium horridum Malme	x								
Eryngium zosterifolium H. Wolff	x								
Eupathorium sp.	x								
Eupatorium inulifolium Kunth	x								
Eupatorium nummularium Hook. & Arn.	x								
Euphorbia peperomioides Boiss.	x		x	x	x	x	x	x	
*Galium humile*Cham. & Schltdl.	x	x	x	x	x	x	x	x	x
Gamochaeta americana (Mill.) Wedd.	x			x	x		x	x	x
Gamochaeta coarctata (Willd.) Kerguélen	x								
Gamochaeta pensylvanica (Willd.) Cabrera	x								
Gamochaeta simplicicaulis (Willd. ex Spreng.) Cabrera	x								
Gaylussacia angustifolia Cham.	x			x					x
Glandularia sp.	x								

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Grazielia nummularia (Hook. & Arn.) R.M.King & H.Rob.		x	x	x	x	x	x	x	x
Gymnopogon sp.	x								
Habenaria parviflora Lindl.	x								
Hydrocotyle exigua (Urb.) Malme	x								
Hypericum brasiliense Choisy	x								
Hypericum cordatum (Vell.) N. Robson	x								
Hypochaeris catharinensis Cabrera	x								
Hypochaeris lutea (Vell.) Britton	x								
Hypoxis decumbens L.	x								
Juncus microcephalus Kunth			x	x			x		x
Kyllinga odorata Vahl	x								
Lessingianthus macrocephalus (Less.) H.Rob.	x								
Lessingianthus sellowii (Less.) H. Rob.	x								
Linum erigeroides A. St.-Hil.	x								
Lobelia camporum Pohl	x		x	x	x	x		x	x

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Lobelia nummularioides Cham.	x								
Lomariocycas schomburgkii (Klotzsch) Gaspar & A.R.			x		x		x		
Lupinus rubriflorus	x		x				x		
Luzula ulei Buchenau				x		x			
Lysimachia sp.					x		x		
Macrothelypteris torresiana (Gaudich.) Ching		x	x	x			x	x	x
Mecardonia procumbens (Mill.) Small	x								
Mecardonia tenella (Cham. & Schltdl.) Pennell	x								
Megalastrum connexum (Kaulf.) A.R.Sm. & R.C.Moran		x	x	x		x	x		
Moritzia dasiantha Fresen.	x		x	x	x		x		x
Nassella filicumis (Delile)Barkworth			x				x		
Nassella vallsii (A. Zanin & Longhi-Wagner) Peñail.	x								
Noticastrum decumbens (Baker) Cuatrec.	x								

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Oldenlandia salzmannii(DC.) Benth. & Hook. f. ex B.D. Jacks.	x								
Oxalis articulata Savigny	x								
Oxalis bipartita A. St.-Hil.	x								
Oxalis brasiliensis Lodd., G. Lodd. & W. Lodd. ex Hildebr.	x								
Oxalis sp. 1	x								
Oxalis sp. 2	x								
Paepalanthus catharineae Ruhland	x								
Paronychia chilensis DC.	x								
Paspalum lepton Schult.	x								
Paspalum maculosum Trin.	x								
Paspalum notatum Flüggé	x								
Paspalum pauciciliatum (Parodi) Herter	x								
Paspalum plicatulum Michx.	x		x	x		x		x	x
Paspalum polyphyllum Nees ex Trin.	x		x	x			x		x
Paspalum pumilum Nees	x	x	x	x	x	x	x	x	x
Paspalum umbrosum Trin.			x		x		x		
Perezia squarrosa (Vahl) Less.	x								

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Petunia altiplana Ando & Hashimoto		x			x	x	x		
Pfaffia tuberosa (Spreng.) Hicken	x								
Piptochaetium montevidensis (Spreng.) Parodi	x								
Plantago australis sub. australis	x								
Plantago australis sub. hirtella (Kunth) Rahn	x								
Plantago lanceolata			x				x		
Poaceae sp.	x								
Polygala australis A.W. Benn.	x								
Polygala brasiliensis L.	x								
Polygala campestres Gardner	x								
Polygala linoides Poir.	x			x	x		x		
Polygala pulchella A. St.-Hil. & Moq.	x								
Polygala pumila Norlind	x								
*Polygala sabulosa*A.W. Benn.	x		x	x			x	x	x

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Polygala sp. 1	x								
Polygala sp. 2	x								
Rhynchanthera brachyrhyncha Cham.	x	x	x	x	x		x	x	x
Rhynchospora barrosiana Guagl.	x	x	x	x	x	x	x	x	x
Rhynchospora brasiliensis Boeckeler		x	x	x	x	x	x	x	x
Rhynchospora edwalliana Boeckeler	x	x	x	x	x	x	x	x	x
Rhynchospora globosa (Kunth) C. Presl	x								
Rhynchospora marisculus Lindl. ex Nees	x	x						x	
Rhynchospora sp.	x								
Richardia humistrata(Cham. & Schltdl.) Steud.	x								
Schizachyrium tenerum Nees	x								
Scleria distans Poir.	x								
Scleria sellowiana Kunth	x	x	x	x	x	x	x	x	x
Selaginella sp.	x								
Senecio brasiliensis (Spreng.) Less.	x	x	x	x	x	x	x	x	x
Senecio sp.				x			x		

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Setaria vaginata Spreng.	x								
Sisyrinchium micranthum Cav.	x								
Sisyrinchium palmifolium L.	x				x		x		
Sisyrinchium vaginatum Spreng.	x	x	x	x	x	x	x	x	x
Solanum guaranticum A.St.-Hil.			x				x		
Sorghastrum scaberrimum (Nees) Herter	x		x	x	x		x	x	x
Sphagnum sp.	x								
Sporobolus camporum Swallen	x								
Steinchisma hians (Elliott) Nash	x								
Stenandrium dulce (Cav.) Nees	x								
Stenocephalum megapotamicum (Spreng.) Sch. Bip.	x								
Stevia lundiana DC.	x								
Tibouchina gracilis (Bonpl.) Cogn.	x			x				x	x

Species	Reference area	Substrate levels (f1)				Hay levels (f2)			
		Control	Removal	Scarification	Burn	Control	Sommer Hay	Spring Hay	Sommer & Spring Hay
Trichocline catarinenses Cabrera	x								
Trifolium riograndense Burkart	x								
Verbena hirta Spreng.	x		x	x		x	x		x
*Verbena montevidensis*Spreng.	x								
Wahlembergia linarioides (Lam.) A. DC.	x								
Xyris cf. reitzii				x			x		x

CAPÍTULO 3

Advancing ecological restoration in the Subtropics: five practical guidelines for grasslands restoration

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Advancing ecological restoration in the Subtropics: five practical guidelines for grasslands restoration

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Abstract

Grassland restoration in the neotropical grasslands is a recent theme when compared to restoration of forest ecosystems. Specific restoration techniques for grasslands are still being developed, and the technical and legal side of restoration are still much influenced by experiences from forest restoration. However, as ecosystems differ in terms of the ecological processes, this forest-bias may impede vegetation recovery. Here we discuss five topics that should - based on ecological knowledge of grassland ecosystem - govern the restoration of grasslands from a rather applied perspective, namely: (I) Impede shrub and tree encroachment, (II) Active use of appropriate disturbance to reach grassland conservation and restoration goals, (III) Use grazing animals as disturbance agents, (IV) Management to control invasive species (V) Keep soil fertility levels low. Each topic is discussed to contextualize its importance in maintenance of grasslands communities in neotropical grasslands.

Key-words: grasslands active restoration, disturbances, management, techniques

INTRODUCTION

Ecological restoration has gained visibility and legal support in many countries in the last years (Meli et al., 2017) Its objectives go beyond the recovery of biodiversity. Restoration aims to recover ecosystems services and to contribute to sustainable development, thus compensating damages caused by rampant conversion of natural areas to other land uses (Choi, 2007). These benefits legitimize restoration projects and are at the basis for their sponsorship by a diversity of sectors. Large-scale restoration initiatives such as the Bonn Challenge, or the Working for Water program in South Africa (Gibson & Barrie Low, 2003) are important as they set concrete restoration aims – often related to global aims, such as the Aichi targets – and promote the development and implementation of restoration strategies. Nonetheless, in some of these initiatives, and specifically in the Bonn Challenge, a bias on forests remains and has been subject to critique and debate (Buisson et al., 2018; Temperton et al., 2019; Veldman, Overbeck, et al., 2015) In the tropics, actions such as the Pacto pela Restauração da Mata Atlântica, and Rede de Sementes do Xingu in Brazil, and the Costa Rica Restoration Project, in Costa Rica, have high visibility and strong social sympathy, but are focused on forest systems.

However, tropical, and subtropical ecosystems encompass other plant formations that go beyond forests and whose conservation and restoration likewise require attention in the context of conservation and restoration that has been neglected. Naturally open ecosystems cover around 31-43% of terrestrial surface of the Earth (Gibson 2009) and have significant environmental and cultural value to the world (Parr, Lehmann, Bond, Hoffmann, & Andersen, 2014). With specific ecological characteristics when well conserved, grass-dominated ecosystems are rich in species, endemism, have high conservation value and, therefore, considered old-growth grasslands *sensu* Veldman et al. (2015a). This concept was an important step to synthesize characteristics of these ecosystems and to reinforce their complexity and the species adaptation that make up old-growth grasslands. However, some themes and concepts adjacent to grasslands theme remain unclear and deficient of data and experiences when compared to the experience acquired over time with forest systems.

Which can imply in that techniques and concepts from forest restoration are indiscriminately applied to grasslands.

Tropical and subtropical savannas and grasslands are highly threatened ecosystems (Veldman, Buisson, et al., 2015). Data from Espírito-Santo et al. (2016), Grecchi et al. (2014) and Andrade et al. (2015) show that tropical and subtropical grasslands have been converted for agriculture or urban lands and, this scenario of agricultural expansion should remain due to new production techniques. Recently, some discussions have been brought in terms of conservation needs (Gerhard E Overbeck et al., 2013; Temperton et al., 2019) and about ecological characteristics of grassy biomes that corroborate that these ecosystems are ancient ecosystems, with high morphological adaptability to the disturbances imposed and highly diverse (Buisson et al., 2018; Parr et al., 2014; Veldman, Buisson, et al., 2015).

The permanence and maintenance of old-growth grasslands are, in most cases, disturbance-dependent, due to their evolutionary history development. However, human occupation and land use changes promoted a gradual decrease of natural disturbances (natural fires and herbivory) and give way to other anthropogenic forms of grassland maintenance (Navarro et al., 2015). Ways that can cause confusion as to the impact (positive or negative) of these actions on the grasslands ecological processes. Here, we want to explore some essential concepts for grasslands dynamics that are key elements in the theoretical basis for projects and specific environmental policies specially involving restoration and conservation.

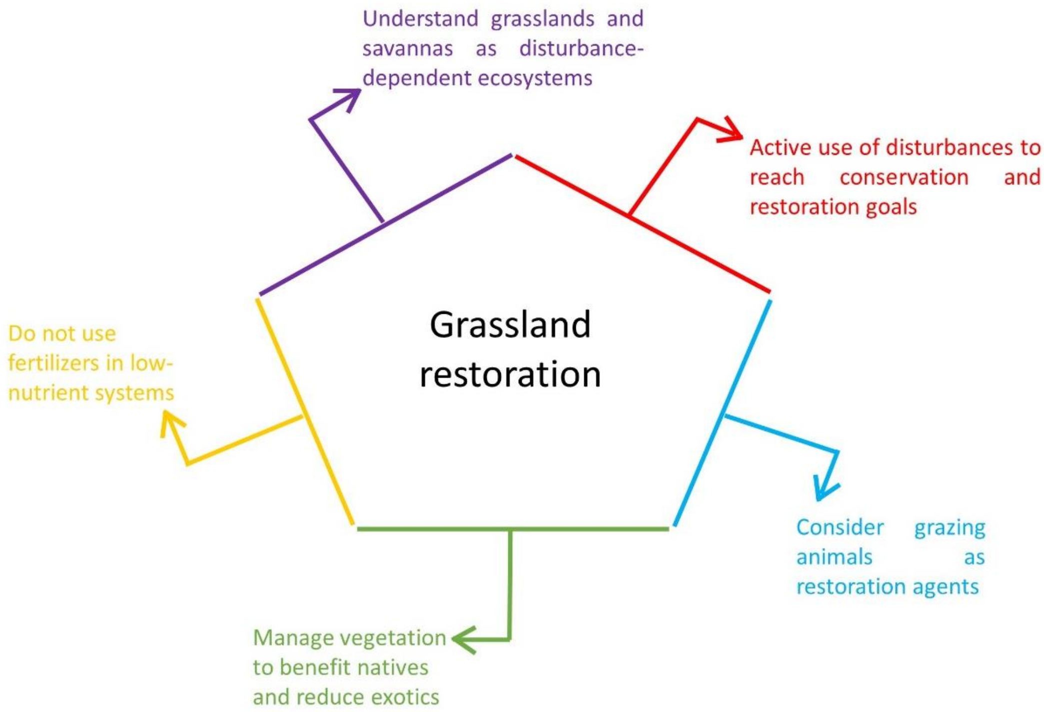

Fig. 1. Five principles to guide restoration of neotropical grasslands.

Impede forest succession processes

In forest ecosystems, the aim of restoration is to facilitate successional processes towards a climax forest community (Christensen, 2014). Indeed, the concept of succession and ecological restoration are deeply linked (Lawrence R Walker, Walker, & Hobbs, 2007) however, the role of succession for restoration of tropical and subtropical grasslands must be seen from a different angle. In passive restoration of forest ecosystems under suitable abiotic conditions and favorable landscape context, the area to be recovered is isolated from disturbances to allow natural or unassisted recovery of forest through secondary succession (Rohr et al., 2016). Differently, natural neotropical grasslands under climatic and soil conditions that allow tree development are maintained at an equilibrium state by disturbances, such as fire or grazing, that impede successional processes towards woodland by periodic, or continuous, reduction of shrub and tree biomass impeding their dominance (Buisson et al., 2018; Veldman, Buisson, et al., 2015). At these ecosystems, several vegetation states composing the grassland predominant matrix are possible which ranging since from prostate grasslands, grasslands composed of prostrate and tussock grasses, and shrubs, and mostly shrubs, varying according to frequency and intensity of these disturbances. In grasslands, a passive restoration approach, as advocated for many in forest systems (Crouzeilles et al., 2017), will promote a vegetation trajectory with a different course from the original composition present before the damage. Generating a biomass accumulation and shrub encroachment, distancing the new area composition from the purpose of restoration and conservation of original vegetation (Cava et al., 2018).That is, for restoration with purpose of grassland and savanna conservation in tropical and subtropical systems, succession management should be considered as a strategy to control biomass accumulation and promote the equilibrium among vegetation recovery, biomass production and biodiversity (Guo, 2007). When restoring most subtropical grasslands, we must recognize that these are not systems that will reach their original (grassland) as final vegetation state if they follow the succession determined by the environment and yes, a woody majority physiognomy. In contrast, to maintain their specific biodiversity, biotic interactions, and other characteristic features, the process of spontaneous succession needs to be impeded by appropriate management that stabilizes the grassland community.

Active use of disturbances to reach grassland conservation and restoration goals

Seen as villains in forest ecosystems due to their negative impact, mainly on regenerating component of the forest (Carlucci, Luza, Hartz, & Duarte, 2016; Chazdon, 2003), disturbances, such as fire and grazing, are allies in active restoration processes in grasslands. However, their role as a conservation tool for neotropical subtropical grasslands has been stigmatized for a long time in South American grassland ecosystems. Only recently acceptance for the need of disturbances is rising on Cerrado biome (Cava, Pilon, Ribeiro, & Durigan, 2018; Durigan & Ratter, 2016). However, the influence of romanticized vision of natural ecosystems as systems without any human influence that exists in environmental policies and even by managers of protected area (Gerhard E Overbeck et al., 2013) makes it difficult to deconstruct long ingrained ideas of conservation that often do not reflect sufficiently their own objectives (Gerhard Ernst Overbeck, Ferreira, & Pillar, 2016; Sarkar, 1999).

Yet, many remaining old-growth grasslands are often degraded exactly by alterations in frequency of disturbances regime. In their decrease or absence, suffering with woody species encroachment (Buisson et al., 2018; Liu et al., 2013). In many grassland regions, at least under productive climate conditions, the consequence of disturbances exclusion is the dominance of the plant community by a small number of tall tussock grasses and shrubs, resulting plant species loss and changes in plant community composition and physiognomy (W. J. Bond, 2016; Fidelis, Blanco, Müller, Pillar, & Pfadenhauer, 2012; Gerhard Ernst Overbeck, Müller, Pillar, & Pfadenhauer, 2005; Veldman, Buisson, et al., 2015), as well as in losses of other grasslands species groups (Abreu et al., 2017). Fire and grazing, by removing biomass and lowering vegetation height, maintain the typical structure, plant diversity and physiognomy of grassy ecosystems (William J Bond & Keeley, 2005) whose species have evolved with these disturbances and are adapted to them (Ripley et al., 2015; Veldman, Buisson, et al., 2015). In Cerrado, restrictive policies regarding fire use results in biodiversity loss (Durigan & Ratter, 2016; Fidelis, 2020). Similarly, in the Campos Sulinos region, biodiversity loss is reported in protected areas where fire and grazing are excluded (Pillar & Vélez, 2010). Even vertebrate species of open habitats depend of a regular disturbance regime (Bencke, 2009). This said, it needs to be emphasized that intensities and frequencies of these disturbances in different non-forests ecosystems cannot be generalized, but may be quite distinct among different climatic conditions, community types and productivity levels (William J Bond & Keeley, 2005; Fidelis & Pivello, 2011).

Thus, when working with active management in grassland and savanna conservation and restoration, we must be aware that the type of management (e.g. fire, grazing, fire and grazing) and its regime (i.e. frequency, intensity,) will be decisive to shape plant community and, consequently, the habitat for other species (Guo, 2007; Lehmann & Parr, 2016). As both fire and grazing are part of the evolutionary history of grasslands and savannas in the Tropics and Subtropics, we can define different target systems, depending on desirable vegetation structure or species composition, or either depending on the socio-economic context. For example, grassland restoration on privately owned properties can aim at the re-establishment of grasslands that can be grazed by cattle and bring economic returns (Liu et al., 2013). On the other hand, in protected areas or in regions where no grazing animals are available, prescribed fires may be more feasible and, exceptionally, despite the costs, even periodic mowing may be an option, e.g. in urban settings where fires are problematics and no grazing animals are available. These different options for vegetation and ecosystem management depend on the objectives set for the specific restoration project, which in turn are influenced by the specific socio-economic context (e.g. Bullock et al., 2011). In practical terms, this gives flexibility and allows for the much called-for stakeholder engagement. On a regional scale, the combination of different restoration and conservation strategies should maximize biodiversity conservation and restoration.

Grazing animals as restoration agents

While both fire and grazing animals can be important in the restoration process to control biomass and vegetation dynamics, grazing animals can also contribute to overcome seed limitation. Animals can be actively used to bring in seeds into areas under restoration by transporting seeds in their digestive system (endozoochory; Treitler et al., 2017) and attached to their fur or hooves (ectozoochory; Römermann et al., 2005). Seed dispersal by animals can be especially important in regions where seed limitation is a major challenge for grassland restoration, and in which availability of commercially obtainable seeds still is low. In neotropical grasslands, no native grazers are found in large herds that can be used in restoration management.

Exotic animals such as beef cattle, sheep or horses can be used as restoration agents although, their use in conservation policies still considered a taboo, especially in areas of full protection (de Patta Pillar & Vélez, 2010; Gerhard Ernst Overbeck et al., 2016). Although, in conservation units with sustainable use category or in private areas with a legal need for restoration, the combining of introduction of seeds for recovery by animals with economic interest, and adequate management between conserved areas (propagule sources) for degraded areas promoting seed input, productivity, and sustainable use of grasslands (Kemp et al., 2013) can be the perfect synergy for joining long-term recovery and validation projects.

The use of grazing animals as restoration agents can bring benefits that go beyond their use to control biomass and to disperse seeds: Domestic grazers that can bring commercial benefits (e.g. production of meat, leather and wool) that allows for conservation and restoration with economic benefits through proper grasslans managemet. It is well known that high grazing levels, i.e., overgrazing have negative consequences for biodiversity and productivity (e.g. Mysterud, 2006) and intermediate intensity contributes the non-degradation of the grasslands (Fedrigo et al., 2018; Liu et al., 2013). This should be especially relevant during the restoration process, as plant community may be more sensitive in early recovery phases, and as risk of spread of invasive species can be high. Regular monitoring of vegetation development and adaptive management, with periodic and strategic grazing exclusions, are key on grassland recovery (Fedrigo et al., 2018). Overall, the use of grazing animals in grassland restoration can contribute, in some types of grasslands, to re-installment of characteristic ecological processes of this ecosystems (Papanastasis, 2009; Rosenthal, Schrautzer, & Eichberg, 2012; Schaich, Szabó, & Kaphegyi, 2010), and also involve local people. Allowing, consequently, the success and economics benefits of the project at long-term (Perring et al., 2015).

Management to control invasive species in areas under restoration

Exotic species invasions have increased considerably in the last decades and are one of the main degradation factors of natural areas (Pyšek & Richardson, 2010). Their consequences transcend biological issues, and they may also lead to negative economic impacts for private property owners or public areas. The invasion of exotic species can be the main degradation factor in an area, or be a consequence of other degradation processes, as mining or intensive agriculture (Lemke, Schweitzer, Tadesse, Wang, & Brown, 2013; Macdougall & Turkington, 2005).

Just as in forest restoration, strategies of population control and propagule pressure control are basic actions for native vegetation recovery where invasive species are present. However, if the invasion is a consequence of a degradation process, recovery strategies needed can be more complex due to possible indirect effects of exotic species and removal actions (Zavaleta, Hobbs, & Mooney, 2001). A difference can be made regarding the functional type of the invasive plant species: Invasion of woody species, such as Pine trees and gorse in grasslands (León Cordero, Torchelsen, Overbeck, & Anand, 2016; Zalba & Villamil, 2002) are problematic and not easy to control, but can be more easily perceived in th early stages of colonization, what can facilitate the invasion control by punctual actions. With an active approach, as removal this functional type not belonging to the grasslands ecosystems and attempt to reestablish native species in situation where they had been suppressed, e.g. after severe invasion. The challenge can be greater in the case of invasion by grasses or herbs, i.e., species that belong to functional categories that also build the community to be restored. Here, the inicial colonization may go unnoticed due to similarity with the landscape-forming plant matrix. In this case, biomass management should be focused to favor of native species and reduce exotic components. For this, grazing (see above), mowing, and fire may be helpful tools, if used with great care, with timing and intensity planned to consider phenological cycles of invasive species and native species community. The problem is that disturbances can also favor invasive species once they often present advantages over native species that allow their spread (Baruch & Bilbao, 1999; D'Antonio & Vitousek, 1992; Williams & Baruch, 2000). For example, at Cerrado, the invasive grasses *Urochloa brizantha* and *U. decumben*s are well adapted to fires which favors them to occupy the burned area in detriment to native species (Gorgone-Barbosa et al., 2016). In the Campos Sulinos, *Eragrostis plana* is a widely spread unpalatable invasive grass that is avoided by cattle and consequently is indirectly benefitted by grazing.

Where fertilization goes against vegetation recovery

As consequence of changes in environmental legislation and socio-economic aspects, the increase of abandoned agricultural lands world-wide is currently a driver of degradation which generates high demand for recovery (Hobbs et al., 2006). These old fields often have a legacy of fertilized soils, when compared to natural or semi-natural grasslands. Nutrient enrichment by fertilization or other anthropogenic inputs, such as Nitrogen deposition, can lead to changes in original soil microbiota, plant biomass increases, and consequently increased competitiveness, and facilitate plant exotic species invasions (Bissett, Brown, Siciliano, & Thrall, 2013; Brooks, 2003). Thus, for restoration of natural grasslands, it is necessary to reduce nutrient levels in the soil. Topsoil removal has been shown an effective intervention to reduce nutrient contents, principally in Central and Western Europe (Allison & Ausden, 2004; Török, Vida, Deák, Lengyel, & Tóthmérész, 2011). In some cases, repeated biomass mowing and removal, over longer periods, has also proved be efficient to remove nutrients from the system (Maron & Jefferies, 2001). Other alternative is carbon addiction in the soil, which promotes an inducing immobilization of plant-available nitrogen (Blumenthal, Jordan, & Russelle, 2003).

These practices contrast with those usually done in forest restoration: as a rule, fertilization with chemical or organic fertilizers is recommended in plantings of tree species (Neto, Siqueira, Curi, & Moreira, 2000). The conceptual difference seems to be that in forest restoration – at least when using plantings – we are, in the first years of restoration project, very much concerned with survival of the individuals that had been planted, while in restoration of grasslands the perspective is much more on the population and community level, and where (see above) much evidence for the negative effects of high nutrient levels exists.

CONCLUSIONS

With high conversion rates in subtropical and tropical grasslands, understanding how the ecological processes that shape grassland recovery can be used as a tool to restoration active practices is key to advance in restoration projects in large scale in neotropical grasslands. In tropical and subtropical regions, forest restoration is much more advanced in terms of techniques and experiences, while grassland restoration has only recently become an important issue (Gerhard E Overbeck et al., 2013; Pilon et al., 2018). There are still many challenges, specifically the lack of herbaceous species seeds, lack of restoration techniques experiences and lack of knowledge on successional pathways after degradation (Pilon et al., 2018; Thomas et al., 2018). With the deficiency of data and experiences, one risk is that techniques and concepts from forest restoration are indiscriminately applied to grasslands. While ecological principles and theories such as succession, facilitation, plant invasion can be applied to all kinds of ecological systems, working with them in a way to restore degraded ecosystems may require quite different, often contrasting, approaches. Here we seek to clarify important points for the advancement of restoration and conservation grassy ecosystems. To achieve good restoration results, taboos such as the use of domestic grazers in restoration need to be demystified, and restoration concepts need to be free of forest bias. Only approaches that correspond to ecological conditions of tropical and subtropical grasslands can serve for the development of specific public policies and related legal requirements. Finally, we would like to stress that, in regions where environmental conditions are adequate, the use of cattle allows to align grassland restoration with economic return project development by means of an extensive livestock, promoting the permanence and validation of the restoration project.

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CONSIDERAÇÕES FINAIS

Esta tese explorou uma área, até então sem experiências nos campos do sul do Brasil, que foi o teste de técnicas de restauração para remoção de serapilheira e introdução de sementes. Acreditamos que com esta experiência e com os resultados obtidos, ao longo destes mais de quatro anos na área de restauração, amadurecemos muito na compreensão do funcionamento de áreas degradadas e principalmente, das limitações que permeiam projetos de restauração de ecossistemas campestres.

Através do diagnóstico do banco de sementes foi possível observar a limitação da regeneração da comunidade a partir do banco de sementes do solo. Na parte experimental do projeto, obtivemos resultados importantes para os fatores testados. O incremento de riqueza de espécies por inserção de feno, e a influência limitadora que a serapilheira tem para o reestabelecimento da vegetação e as consequências da manipulação do substrato, que proporcionou um estímulo do banco de sementes composto por poucas espécies da família Cyperaceae, formando comunidades com cobertura vegetal muito distinta da área de referência, indicando um possível “priority effect”.

Ao longo do tempo de pesquisa fomos percebendo o quanto algumas práticas de restauração campestre e manejo não são bem compreendidas, e por isso não são bem vistas, por órgãos públicos, gestores e pela sociedade em geral. E a partir desta experiência buscamos no capítulo três explorar temas que são chave para o avanço na discussão de restauração de ecossistemas campestres tropicais e subtropicais

Nossa pesquisa traz uma experiência importante no âmbito da restauração, mas de certa forma também indica que o caminho ainda é longo para a recuperação de áreas em escala de paisagem quando não temos ainda sementes ou mudas disponíveis para a comercialização. É imprescindível avançar ações e subsídios que estimulem produtores e coletores de sementes, para que projetos de restauração possam se realizar em escalas largas. Através das experiências vividas e da aprendizagem que a restauração ecológica vem proporcionando, a maior lição é que conservar áreas naturais ainda é o melhor caminho.