UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Tese de Doutorado

Restauração ecológica de ecossistemas campestres: técnicas e perspectivas aplicadas aos Campos Sulinos

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Porto Alegre, novembro de 2023

RESTAURAÇÃO ECOLÓGICA DE ECOSSISTEMAS CAMPESTRES: TÉCNICAS E PERSPECTIVAS APLICADAS AOS CAMPOS SULINOS

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Doutor em Ecologia.

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Porto Alegre, novembro de 2023

CIP - Catalogação na Publicação

Thomas, Pedto Augusto
Restauração ecológica de ecossistemas campestres: técnicas e perspectivas aplicadas aos Campos Sulinos / Pedto Augusto Thomas. -- 2023.
139 f.
Orientadora: Sandra Cristina Müller.

Coorientador: Gerhard Ernst Overbeck.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Programa de Pós-Graduação em Ecologia, Porto Alegre, BR-RS, 2023.

1. Ecologia da Restauração. 2. Campos Subtropicais. 3. Espécies invasoras. 4. Atributos Funcionais. 5. Fogo. I. Müller, Sandra Cristina, orient. II. Overbeck, Gerhard Ernst, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Agradecimentos

Tornar-me doutor em ecologia é resultado da colaboração de diferentes formas de várias pessoas ao longo desses quatro anos e meio. Eu agradeço muito a todas elas.

Aos amigos do Ronã, do Danone, de outros grupos, e também aos sem grupo, que ajudam a tornar os dias de doutorado mais leves, independente da frequência dos encontros.

Aos amigos e colegas de Leveg e Levcamp, por tantos cafés, campos e trocas de ideais. Mas em especial à Ana e ao Filipe, com quem fiz tantos campos junto ao longo desse doutorado. Também à Alice, que me ajudou muito durante meus experimentos na pandemia e tive a sorte de virar co-orientador do TCC.

À Capes pela bolsa de doutorado e ao CNPq pela bolsa de doutorado-sanduíche. Também ao Funbio e Instituto Humanize pela Bolsa Funbio que permitiu que eu fizesse meus campos.

À Sandra e ao Gerhard, meus orientadores, por toda a paciência e ensino ao longo desses 4 anos e meio (+ 2 anos de mestrado).

À Elise Buisson por me receber tão bem em Avignon. E também aos amigos franceses, italianos e brasileiros que fiz por lá. Um abraço especial pra Augusto, Remy e Lia, que tornaram os dias em Avignon mais leves depois de tanto trabalho.

Aos funcionários do Parque Nacional da Lagoa do Peixe, do Parque Natural Municipal Saint'Hilaire e do Jardim Botânico de Porto Alegre que tanto me ajudaram durante os campos e os experimentos.

À minha irmã Bruna, meu cunhado Carlos, minha mãe Rosani, e meu pai Sidnei, lá em Arroio do Meio, onde pude ir menos vezes do que todos nós gostaríamos.

E por fim, à Tali, meu amor, por todo carinho, paciência e companheirismo ao longo desses anos de doutorado e namoro que praticamente começaram juntos.

SUMÁRIO

RESUMO

Os ecossistemas campestres ao redor do globo contêm uma grande biodiversidade e provêm diversos serviços ecossistêmicos. Esta realidade não é diferente para os Campos Sulinos, no sul do Brasil. Entretanto estes campos estão fortemente ameaçados por, principalmente, conversão de hábitat e espécies invasoras. A partir disso, a restauração ecológica é fundamental para os Campos Sulinos, mas experiências de restauração são poucas e recentes, e existem muitas lacunas no conhecimento. Diferentemente, os ecossistemas campestres temperados do Hemisfério Norte têm um longo histórico de restauração, e este conhecimento disponível pode ajudar a desenvolver a restauração aqui. Nesse contexto, essa tese o objetivo geral de contribuir para o desenvolvimento da restauração ecológica nos Campos Sulinos, a partir de capítulos teóricos e experimentais. O primeiro capítulo é um estado da arte da restauração ecológica na região, descrevendo também as características e ameaças aos Campos Sulinos, bem como desafios e caminhos futuros para a sua restauração ecológica. Os dois capítulos seguintes são experimentos realizados em casa de vegetação. No primeiro foi avaliado o potencial do feno, técnica promissora para a restauração na região, de introduzir sementes de espécies nativas quando coletado em diferentes períodos do ano e aplicado em duas quantidades. O experimento demonstrou que o feno tem potencial para introduzir espécies nativas de graminóides, porém há uma grande variação no número de plântulas dependendo da época e local de coleta. No segundo experimento criamos comunidades para competir com a invasora *Eragrostis plana*. Usamos uma abordagem de ecologia funcional para definir as comunidades de espécies nativas a partir de atributos funcionais foliares, e as comunidades foram semeadas com densidades diferentes. Os resultados demonstraram que desenhar comunidades a partir de uma perspectiva funcional não é eficiente para competir com a invasora, enquanto aumentar a densidade de sementes de espécies nativas sim. E o último capítulo foca no uso do fogo, um distúrbio endógeno em diversos ecossistemas campestres, como ferramenta de restauração ecológica. Neste capítulo fizemos uma revisão bibliográfica para saber se e em quais situações o fogo é útil na restauração. A revisão mostrou que em diversas situações o fogo é positivo. Porém na maioria das situações o seu efeito é nulo, e o resultado varia conforme o ecossistema campestre, o objetivo e as técnicas utilizadas. Por fim, essa tese demonstrou alguns caminhos para impulsionar o desenvolvimento da restauração ecológica dos Campos Sulinos. Mas fica claro que muitas dúvidas permanecem e mais estudos experimentais, principalmente com maior escala espacial e temporal, são necessários. A restauração ecológica é fundamental para os Campos Sulinos e deve ser expandida.

PALAVRAS-CHAVE: Atributos funcionais, Campos subtropicais, Ecologia da restauração, *Eragrostis plana*, Espécies invasoras, Fogo, Pampa, Transposição de feno

ABSTRACT

Grassland ecosystems around the world have a high biodiversity and provide several ecosystem services. This reality is not different from the Campos Sulinos grasslands, in Southern Brazil. Nevertheless, these grasslands are strongly threatened by, mainly, habitat conversion and invasive species. In this context, ecological restoration is an important activity for the future of Campos Sulinos. However, restoration experiences began in the last decade in the region, and several knowledge gaps remain. Contrarily, temperate grasslands in the North Hemisphere have a long history of restoration based on scientific research, and this available knowledge can be helpful in developing the restoration here. In this context, this thesis has the main objective of contribute to the development of restoration ecology in Campos Sulinos, with both theoretical and practical chapters. The first chapter is a state of the art of the ecological restoration in Campos Sulinos, also discussing its main characteristics and threats, as well as the challenges and future ways forward. The next two chapters are experiments developed in greenhouse. In the first one, we assessed the potential of hay transfer, a promising technique for the region, to introduce native species when collected on different dates and applied with different hay amounts. The experiment showed that hay has potential to introduce native graminoids, however, there is great variation regarding the number of seedlings according to the date of harvest and hay donor site. In the second experiment, we sowed native plant communities to compete with the invasive *Eragrostis plana*. From a functional approach, we designed communities based on leaf functional traits, and the native communities were sowed with three different seed densities. The results showed that design communities based on functional traits is not efficient to compete with the invasive species, but increasing the native seed density is a good approach. The fourth chapter focused on the use of fire, an endogenous disturbance in several grassland ecosystems, as a tool for ecological restoration. We made a systematic review to know if and when fire is a helpful tool. The review shows that in several situations fire has a positive effect on restoration. Nevertheless, in the majority of situations fire has no effect, and results can vary according to the grassland ecosystem, the objective, and the technique employed. This thesis demonstrated some ways to boost the develop of the ecological restoration in the Campos Sulinos. Nevertheless, it is clear that several gaps remain, and more experiments are needed, mainly with larger temporal and spatial scales. The ecological restoration of Campos Sulinos is fundamental and must be expanded.

KEY WORDS: *Eragrostis plana*, Fire, Functional traits, Hay transfer, Invasive species, Pampa grasslands, Restoration ecology, Subtropical grasslands,

INTRODUÇÃO GERAL

Ecossistemas campestres são ecossistemas dominados por gramíneas e outras espécies herbáceas e cobrem aproximadamente um terço da superfície terrestre (Bond & Parr 2010; Gibson 2009). Estes ecossistemas são associados com uma grande biodiversidade, mas também fornecem vários serviços ecossistêmicos importantes, como sequestro de carbono, fornecimento de água, e produção de forragem para a pecuária (Bengtsson et al., 2019; Zhao et al., 2020). No entanto, a conservação dos ecossistemas campestres tem sido fortemente ameaçada. Conversão de habitat é a principal razão histórica de perda de biodiversidade (Gibson, 2009; Petermann & Buzhdygan, 2021), mas outras ameaças como invasão biológica, alterações nos regimes de distúrbios, e mudanças climáticas também são grandes ameaças (Gibson 2009; Buisson et al., 2019; Veldman et al., 2015). Neste contexto, a sua restauração ecológica é extremamente importante para garantir a conservação da biodiversidade e a manutenção dos serviços ecossistêmicos (Clewell & Aronson, 2006; IPBES, 2018).

No sul do Brasil, a realidade dos Campos Sulinos não é diferente. Estes campos subtropicais estão entre os ecossistemas campestres mais diversos do mundo (Andrade et al., 2019; Overbeck et al., 2007), com uma riqueza estimada de 3000 espécies de plantas (Boldrini et al., 2015). Estes ecossistemas são heterogêneos e compreendem os campos dos biomas Pampa e Mata Atlântica nos três estados da região sul do Brasil, e possuem incalculável valor cultural para a população (Vélez-Martin et al., 2015). A figura do gaúcho e o churrasco são elementos importantes da cultura do sul do Brasil, assim como na Argentina e no Uruguai, e têm origem na vida e no trabalho sobre estes ecossistemas campestres. A pecuária sobre campo nativo é uma importante atividade econômica na região e possui grande potencial para a produção sustentável (Valls et al., 2009).

A conservação da vegetação original e da paisagem dos Campos Sulinos são, então, importantes em termo ecológicos, culturais e econômicos. Apesar disso, aproximadamente 60% da cobertura dos Campos Sulinos já foi convertida para outros usos, principalmente para agricultura (Vélez-Martin et al., 2015). E essa conversão de campo nativo tem se intensificado nas últimas duas décadas. Além da conversão de habitat, a presença de espécies invasoras é outra grande ameaça a esses ecossistemas (Guido & Guadagnin, 2015). Dentre as espécies de plantas invasoras, destaca-se a gramínea C4 *Eragrostis plana* Nees, o capim-annoni. A invasão por *E. plana* é um problema não só em áreas previamente convertidas, mas também naquelas tiveram um manejo inadequado do gado (Guido & Guadagnin, 2015). Além de ameaçar a biodiversidade, a invasão por *E. plana* causa prejuízos para a produção pecuária, pois essa espécie tem baixo valor nutricional (Guido & Guadagnin, 2015; Medeiros et al., 2009).

O aumento dos impactos humanos nos ecossistemas, como a conversão de hábitat e invasão por espécies exóticas, resultou no interesse em restaurar áreas degradadas para minimizar tais impactos, principalmente sobre espécies e paisagens (Choi et al., 2008; Holl, 2023). Entende-se que proteger áreas conservadas já não é suficiente, sendo também necessário restaurar aquelas que foram degradadas (Fischer et al., 2021; Gann & Lamb, 2006). Neste contexto surge a restauração ecológica, entendida como o processo de auxiliar a recuperação de um ecossistema que foi degradado, danificado ou destruído (Society for Ecological Restoration International, 2004). A restauração ecológica tem se tornado cada vez mais importante para manter o funcionamento dos ecossistemas, a prestação de serviços ecossistêmicos e a conservação da biodiversidade (Holl, 2023; IPBES, 2018), tanto que a ONU considerou a década de 2021-2030 como a década da restauração ecológica.

A partir desse contexto, a restauração ecológica nos Campos Sulinos é fundamental para a conservação da sua biodiversidade e a manutenção da provisão de seus serviços ecossistêmicos. No entanto, experiências e estudos de restauração ecológica na região são poucos e recentes (Overbeck et al., 2013; Overbeck & Müller, 2018), e o debate é praticamente inexistente na sociedade. Testar e aprimorar técnicas de restauração ecológica para a região é prioritário (Overbeck et al., 2013; Vieira & Overbeck, 2015), mas ainda há várias lacunas que precisam ser respondidas para impulsionar a restauração ecológica nos Campos Sulinos. Nesse contexto, está o primeiro capítulo dessa tese, onde apresentamos um panorama geral da restauração ecológica nos Campos Sulinos, apontando as principais ameaças à sua conservação, aspectos legais para a restauração, um estado da arte dos estudos práticos já realizados, e uma discussão sobre fatores limitantes e maneiras de para sobrepô-los.

Em restauração ecológica, normalmente o primeiro objetivo, uma vez cessada a ação degradadora, visa introduzir ou aumentar a abundância de espécies nativas localmente extintas (Buisson et al., 2019). É importante que espécies nativas recubram rapidamente uma área degradada para diminuir a erosão do solo e evitar a entrada de espécies invasoras, por exemplo. Em ecossistemas campestres subtropicais, como os Campos Sulinos esse é um passo fundamental devido ao baixo potencial de recobrimento da vegetação a partir do banco de sementes e dos órgãos subterrâneos em áreas que foram convertidas (Fidelis et al., 2014; Vieira et al., 2015). Campos secundários sem ações de restauração normalmente são muito diferentes de campos nativos (ex.: Torchelsen et al., 2019). Neste sentido, é preciso encontrar técnicas que possibilitam o estabelecimento de espécies nativas em áreas degradadas, normalmente a partir de sementes.

Testar e adaptar técnicas de introdução de espécies normalmente utilizadas na restauração dos campos temperados é uma alternativa (Overbeck et al., 2013; Stradic et al., 2014; Vieira & Overbeck, 2015). Nestes ecossistemas, uma das técnicas mais simples, baratas e efetivas é a aplicação de feno (Kiehl et al., 2010; Török et al., 2011), que consiste em cortar e coletar a vegetação herbácea quando as plantas estão dispersando seus propágulos e aplicar esta biomassa no local que se pretende restaurar. O seu uso é interessante no contexto dos Campos Sulinos também porque não há sementes de espécies nativas em quantidade e variedade disponíveis no mercado para utilização em projetos de restauração (Rolim et al., 2022).

Contudo, há diferenças entre os campos temperados e os Campos Sulinos que devem ser consideradas quando pensarmos no uso da técnica da aplicação de feno. No sul do Brasil, o inverno é mais curto e menos intenso, a vegetação possui uma composição funcional diferente (com maior proporção de gramíneas C4 do que C3) e um longo período de frutificação das espécies nativas, de outubro a abril, com fenologia diferente entre os grupos funcionais de plantas (Boldrini et al., 2015; Overbeck et al., 2018). Essas diferenças mostram que simplesmente replicar a técnica tal qual é empregada em campos temperados pode não ser suficiente e sua eficiência pode variar dependendo de quando for empregada. Estudos recentes que testaram a transposição de feno tiveram resultados diversos que impedem generalizações para toda a região (Porto et al., 2022; Thomas et al., 2019a, 2019b). Testar o feno coletando em diferentes períodos do ano e aplicá-lo em diferentes quantidades nos permitirá saber quando a técnica é mais eficiente e aperfeiçoá-la às características locais. Este é o tema principal do segundo capítulo.

Como já dito, espécies invasoras também são problemáticas nos Campos Sulinos. Ambientes degradados, além da baixa cobertura de espécies nativas, frequentemente estão invadidos por espécies exóticas, sendo esse um dos maiores desafios em projetos de restauração (Buisson et al., 2019; Funk et al., 2008; Suding et al., 2004) e cujo controle deve ser prioridade (Meyerson & D’Antonio, 2002). Um ponto de atenção é que ações para controlar uma invasora, ao liberar recursos, podem criar oportunidades de estabelecimento para a mesma ou outra espécie invasora (Meyerson & D’Antonio, 2002).

É fundamental conseguirmos, simultaneamente, controlar as invasoras e adicionar espécies nativas, para que estas utilizem os recursos liberados (como luz e nutrientes) e diminuam a chance de uma nova invasão. Neste contexto, conhecer os atributos funcionais das espécies é uma abordagem interessante.

Atributos funcionais são características morfológicas, fisiológicas e fenológicas relacionadas ao desempenho das plantas (Violle et al., 2007). Tais informações são importantes, pois conhecendo os atributos funcionais das espécies poderemos introduzir na área degradada aquelas com maiores probabilidades de se estabelecer rapidamente e, assim, atingir mais facilmente os objetivos (Funk et al., 2008; Grman et al., 2015; Laughlin, 2014; Torrez et al., 2017; Yurkonis, 2013). E a partir dos valores similares dos atributos funcionais, podemos selecionar espécies nativas que sejam potenciais competidoras fortes à invasora, promovendo exclusão competitiva da mesma por similaridade limitante (Funk et al., 2008; Hulvey & Aigner, 2014; Laughlin, 2014).

Embora os impactos negativos da invasora *E. plana* sejam conhecidos desde os anos 1970, principalmente na pecuária (Medeiros et al., 2009), poucos estudos de restauração ecológica focaram nesta espécie. Estudos de controle de *E. plana* tem um forte viés produtivo, normalmente semeando espécies exóticas forrageiras para competir com a invasora (veja Medeiros & Focht, 2007). Apenas um estudo testou a semeadura de espécies nativas (juntamente com exóticas) para reduzir o impacto de *E. plana* (Medeiros et al., 2011). Com o uso da ecologia funcional, podemos selecionar comunidades de espécies nativas para competir com *E. plana*. Este é o tema principal do terceiro capítulo.

Ainda no tema da pecuária, o fogo é uma ferramenta usada em várias regiões dos Campos Sulinos para renovar o pasto e “limpar” o campo (diminuir a presença de arbustos), melhorando a qualidade da forragem para o gado, principalmente nos Campos de Cima da Serra e na Serra do Sudeste (Overbeck et al., 2018; Pillar et al., 2010). Os Campos Sulinos coevoluíram com o fogo e plantas nativas apresentam uma série de adaptações, como a presença de gemas protegidas, órgãos subterrâneos de rebrote, e florescimento estimulado pós-fogo (Fidelis & Pivello, 2011). Por isso, o fogo pode ser considerado distúrbio endógeno nos Campos Sulinos, ou seja, é um elemento importante na manutenção da estrutura, dinâmica e biodiversidade destes campos (Overbeck et al., 2007). O mesmo ocorre em outros ecossistemas campestres ao redor do mundo (Bond & Keeley, 2005; Buisson et al., 2019; Veldman et al., 2015).

Assim, como importante elemento na modelagem de ecossistemas campestres, o fogo também pode ser entendido como uma ferramenta na restauração ecológica (Török et al., 2021). No entanto, obviamente, os efeitos e a importância do fogo em ecossistemas campestres variam com a latitude e o clima (Gibson, 2009). E o fogo ainda é visto como controverso e negativo em diversos ecossistemas campestres onde é, na verdade, um elemento importante do seu funcionamento (Silveira et al., 2020). Entender se e como o fogo funciona como ferramenta de restauração ecológica em ecossistemas campestres é importante para promover a restauração destes ecossistemas, incluindo os Campos Sulinos, onde há apenas uma experiência de uso de fogo na restauração (veja Porto et al., 2022). Esse é o tema central do quarto capítulo.

Assim, essa tese, feita com a contribuição de colegas e professores da UFRGS e Avignon Université, tem o objetivo geral ajudar no desenvolvimento da restauração ecológica nos Campos Sulinos, discutindo perspectivas e técnicas. Como já dito, a tese é estruturada em quatro capítulos: no primeiro apresentamos um panorama geral da restauração ecológica nos Campos Sulinos. O segundo foca na avaliação do potencial da transposição de feno como uma técnica para introduzir espécies a partir de um experimento em casa de vegetação. No terceiro capítulo, também um experimento, testamos diferentes comunidades de espécies nativas semeadas em diferentes densidades, criadas a partir de atributos funcionais, para competir com a invasora *Eragrostis plana*. E o quarto capítulo é uma revisão sistemática do uso do fogo na restauração ecológica de ecossistemas campestres ao redor do globo, com potencial de aplicação para os Campos Sulinos.

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CAPÍTULO 1

Ecological Restoration of *Campos Sulinos* Grasslands

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Capítulo do livro "*South Brazilian Grasslands: ecology and conservation of the Campos Sulinos*" (2023) Gerhard Ernst Overbeck, Valério de Patta Pillar, Sandra Cristina Müller & Glayson Ariel Bencke (Eds.). Springer (no prelo).

Chapter 20

Ecological Restoration of Campos Sulinos Grasslands

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Abstract

Ecological restoration is an important activity to ensure biodiversity conservation and the provision of ecosystem services. It has been a growing field in the last two decades in Brazil, but mainly focused on forest ecosystems. The subtropical *Campos Sulinos* grasslands also experienced a growth in restoration studies, but considerably smaller and more recent. Restoration of grassland has fundamental differences from forest restoration due to intrinsic differences between these ecosystems. Subtropical grasslands have high resilience to endogenous disturbances (grazing and fire) and, contrary, low resilience to exogenous disturbances, such as land conversion. Given the current rates of land conversion in *Campos Sulinos*, its ecological restoration is urgent and still has a long way forward. In this chapter, we present the main threats to *Campos Sulinos* grasslands and discuss the legal aspects and demands for its restoration. Then, we present the state of the art of ecological restoration in the region, considering the three different scenarios of degradation: inadequate management, invasive species, and land conversion. Later, we discuss the regional main gaps and bottlenecks for ecological restoration and the ways to overcome them. At the end, we present the first results of two different projects that are under development: the test of a new technique to control an invasive grass, and the insertion of local communities in restoration projects. We hope that this material can be useful to boost future studies and projects of ecological restoration in the *Campos Sulinos* grasslands.

Keywords: Exogenous disturbances, Grassland management, Invasive species, PLANAVEG, Species introduction.

20.1 Ecological Restoration and Subtropical Grasslands

Ecological restoration – the process of assisting the recovery of a degraded ecosystem (Society for Ecological Restoration 2004) – has developed to an important field of work in Brazil, and Restoration Ecology, the field of research that underpins it, has equally gained more attention recently. Much of this is related to the activities of the Atlantic Forest Restoration Pact, an initiative that aims to recover 15 million hectares of degraded land in the Atlantic Forest biome by 2050 (Rodrigues 2009). Restoration of non-forest vegetation ecosystems, such as grasslands and savannas that originally cover 27% of Brazil (Overbeck et al. 2022), however, is still less developed (Guerra et al. 2020). Ecological restoration of grasslands in the *Campos Sulinos* region – comprising the grasslands in the states of Rio Grande do Sul (RS), Santa Catarina (SC), and Paraná (PR) (Overbeck et al. 2022) – is recent: the first larger restoration projects only started in the very recent past. Equally, the research field of Restoration Ecology is new for grasslands in the region; the starting point of the debate might be the paper 'Restoration Ecology in Brazil - Time to Step Out of the Forest', published in 2013 (Overbeck et al. 2013).

The fast conversion of *Campos Sulinos* grasslands to other land uses and their low resilience to severe disturbances make the development of restoration techniques urgent. It is now generally accepted – at least in the scientific community – that the protection of conserved areas is not enough to protect the biodiversity and to maintain ecosystem services and functioning: we need to restore degraded areas (Hilderbrand et al. 2005, Gann and Lamb 2006, Fischer et al. 2021). Previous knowledge from other grassland regions, in particular from temperate Northern Hemisphere grasslands, serves as an important basis for research and practice in ecological restoration in the region. Restoration in the *Campos Sulinos* thus does not start from zero, even though it is necessary to develop techniques, tools, and concepts appropriate to the specific conditions and characteristics of these ecosystems (Overbeck et al. 2013, Silveira et al. 2020). Besides the motivation to restore biodiversity, there are other good arguments for restoration (Holl 2023): economic motivations, such as job and income generation, cultural reasons, and, last but not least, legal obligations. Restoring ecosystems also is an ethical question, as it seeks to leave healthier ecosystems for future generations. Due to the urgency of ecological restoration, the United Nations defined 2021-2030 as the Decade of Ecosystem Restoration. Brazil, with the National Plan for the Recovery of Native Vegetation (*PLANAVEG: Plano Nacional de Recuperação da Vegetação Nativa*, in Portuguese, Brasil 2017) has developed its own ambitious restoration aims: until 2030, 12,5 million hectares of degraded land are to be restored.

Strategies for ecosystem restoration can be classified into two main approaches: passive and active restoration (Holl and Aide 2011, Gann et al. 2019). Passive restoration (sometimes called spontaneous regeneration) considers that upon removing the cause of degradation, the potential for natural recovery is high, as the barriers hindering ecosystem recovery are small; thus, there is no need for human interventions. This approach relies on secondary succession and the inherent regeneration capacity of the system after stopping disturbances. Active restoration, however, is necessary when the potential for spontaneous regeneration is absent or low due to strong limitations, such as altered soil conditions, limited dispersal or establishment of target species, and inadequate disturbance regime (Gann et al. 2019). In active restoration, human interventions are used to allow, or at least speed up, the recovery of the ecosystem, ranging from smaller interventions such as changing management and introducing species by planting or sowing to larger interventions with engineering actions, as in the case of degradation by mining. It should be noted, however, that application of the concept of passive restoration, often successfully applied in forest restoration (Crouzeilles et al. 2017), to grasslands is more difficult. Successional processes in *Campos Sulinos* grasslands without any human management lead to woody encroachment and may result in forest expansion (see Müller et al. 2023, in press). Thus, active vegetation management is necessary along the restoration process even when the potential for natural recovery is high.

The decision of which approach is more adequate in a specific case depends on the magnitude of the limitation that prevents the ecosystem recovery after a disturbance, but also on ecological processes such as seed dispersal (Holl and Aide 2011, Perkins and Leffler 2018, Gann et al. 2019). The goals, the resources, and the budget available for the restoration project also are important elements to decide which strategy to take (Holl and

Aide 2011, Jones et al. 2018). Ambitious goals in a short time require more active interventions than less ambitious goals in the long term.

Subtropical grasslands are highly resilient to fire and herbivory. Both are considered to be endogenous disturbances: over evolutionary periods, they have been important for the maintenance of the structure, dynamics, and biodiversity of many, if not most, tropical and subtropical grasslands (e.g., Bond 2016, Buisson et al. 2019, Veldman et al. 2015, Andrade et al. 2023, in press). The reason for this resilience is the presence, in many species, of belowground structures (e.g., rhizomes, lignotubers, xylopodia that form the bud bank in the soil), that allow for plant survival during and recovery of biomass after a disturbance event (Bond 2016, Fidelis et al. 2009, Veldman et al. 2015). Fire and herbivory should thus not be considered direct drivers of degradation processes, unless their frequency or intensity is far out of the range under which the system has evolved. They may even be interesting and necessary tools in grassland restoration (Buisson et al. 2019, Silveira et al. 2020). Moreover, remnant areas of native grasslands that are managed with grazing and/or fire can be used as reference ecosystems that are needed to inform baseline values of target ecosystem characteristics and functioning. In contrast, exogenous disturbances, such as vegetation suppression for use as agricultural land or silviculture and the invasion of alien species, have strong impacts on biotic and abiotic components of subtropical grasslands, especially when they affect belowground structures and processes (Buisson et al. 2019). After such exogenous disturbances, resilience is likely low, and ecological restoration is thus challenging: it requires active interventions, such as restoring soil conditions, introducing native species, and controlling invasive species (Buisson et al. 2021). These activities will be at the center of this chapter. We start with a short overview of the conservation and degradation of *Campos Sulinos* grasslands, discuss the legal and social contexts of their restoration, and then present the state of the art of ecological restoration in the region. In the closing section, we suggest ways forward to advance in the restoration of grasslands in the region.

20.2 Conservation State and Threats of *Campos Sulinos* Grasslands

As mentioned above, fire and grazing are key factors driving ecological processes in the *Campos Sulinos*, and appropriate management is thus of high relevance for the conservation of these grasslands. In general, exclusion of these disturbances leads the community to be dominated by tall tussock grasses and to woody species encroachment, resulting in species-poor plant communities due to drastic losses of the highly diverse forbs component and prostrate grasses (Boldrini and Eggers 1996, Guido et al. 2017, Sühs et al. 2020). After long-term exclusion of disturbances, the bud-bank also diminishes, especially of forbs (Fidelis et al. 2014), which makes the recovery of this component very difficult, even after the reintroduction of management (disturbances). Grasslands where disturbances have been excluded are also more likely to be invaded by exotic woody species, mainly *Pinus* spp. Management exclusion may occur in protected areas: the consequence is standing dead biomass accumulation, herbaceous diversity loss, shrub (and sometimes tree) encroachment (see Müller et al. 2023, in press, and Overbeck et al. 2023, in press, for more details).

If excluding endogenous disturbances has negative effects on grassland biodiversity and structure, excess of disturbances (intensity and/or frequency) are also problematic. Overgrazing increases erosion, decreases forage quality and quantity, decreases species diversity, and can provide opportunities for the invasion of the African grass *Eragrostis plana* Nees (Overbeck et al. 2007, Vélez-Martin et al. 2015), an extremely problematic species in the region (Guido et al. 2016). It has been estimated that, in 2008, *E. plana* already occupied 2.2 million hectares of grasslands in Rio Grande do Sul (Medeiros and Focht 2007). Similarly, a high frequency of fire may favor the spread of the alien legume shrub *Ulex europaeus* L. (Cordero et al. 2016). Other problematic invasive species in *Campos Sulinos* grasslands include the grasses *Cynodon dactylon* (L.) Pers. and *Urochloa* spp., the Asteraceae *Senecio madagascariensis* Poir., and species of the genus *Pinus* (see also Guido et al. 2023, in press). Moreover, wild boar (*Sus scrofa* L.) invasions have been reported for the *Campos Sulinos* region (Sordi and Lewgoy 2017). Initial research indicates severe impacts on vegetation, primarily because wild boar overturns the soil in the areas to feed on belowground plant organs, fungi, and invertebrates.

Beyond inappropriate management and alien species invasion, habitat conversion is the main threat to *Campos Sulinos* grasslands. Circa 60% of the original grassland cover has already been lost, mainly to agriculture and forestry (Vélez-Martin et al. 2015, Oliveira et al. 2017). Only in the Pampa, i.e., the southern portion of the *Campos Sulinos* region, 38% of the original grassland cover was lost between 1985 and 2021 (reduction from 9.3 million hectares to 5.8 million hectares), while the agricultural area almost doubled (increased from 3.8 million hectares to 7.3 million hectares) (MapBiomas v7.0 2022). During the same period, the area with monocultures of exotic trees in the Pampa increased seventeen-fold from 1985 to 2021 (from 42 thousand to 744 thousand hectares; MapBiomas 7.0 2022), aggravating, in the case of *Pinus*, the problem of biological invasions. Land conversion leads to fragmentation and habitat area loss and thus negatively affects the biodiversity of grassland remnants, e.g., by biotic homogenization (Staude et al. 2018). When converted areas are later abandoned, secondary grasslands may develop that differ considerably from primary grasslands, i.e., grasslands that had never been subjected to land conversion (Koch et al. 2016, Leidinger et al. 2017, Torchelsen et al. 2019). In these cases, active restoration is necessary.

20.3 Demands and Requirements for Restoration

The continuous loss of grassland areas, principally in the recent years, is emblematic for the historical bias of conservation on forests (Overbeck et al. 2013), aggravated, in the case of the *Campos Sulinos* region, by low protection levels considering the extent of protected areas (see Overbeck et al. 2023, in press). A somewhat better consideration of grassland ecosystems in Brazilian environmental law is recent: with the Law for the Protection of Native Vegetation (*Lei de Proteção da Vegetação Nativa*, Brasil 12.651/12), in 2012, the consideration of non-forest ecosystems becomes more explicit (Menezes et al. 2021, Porto et al. 2021). This law includes two legal requirements that can significantly impact the demand for restoration: the Legal Reserve (RL, *Reserva Legal* in Portuguese) and the Areas of Permanent Protection (APP, *Área de Preservação Permanente* in Portuguese), that correspond to portions of each private property that must be conserved or restored (Metzger et al. 2019). APPs are to protect riparian corridors, steep slopes, and other sensitive ecosystems; their extent in the landscape thus varies according to geomorphology. The RL constitutes a fixed amount, in the *Campos Sulinos* region, 20% of the property that is to be maintained with native vegetation cover but can be economically exploited in a sustainable way. Exemptions are made for landowners that converted areas for other uses before 2008. Private landowners whose property is not in accordance with these legal requirements, i.e., who have a deficit in RL or APP, need to restore these areas. Despite the potential for significant restoration demands across all Brazilian biomes (Metzger et al. 2019), the implementation of the law poses challenges. Particularly in RS, the state that includes a substantial portion of the *Campos Sulinos* region, there are initiatives – without any scientific bases – to exempt grasslands that have been used as rangelands from the RL requirements (see details in Overbeck et al. 2023, in press).

Just as the conservation of grasslands in the *Campos Sulinos* region (for which a lack of specific protection requirements has been diagnosed; Porto et al. 2021, Rolim et al.

2022), the restoration of grasslands has received little specific attention until today. The national Environmental Regularization Program (PRA, *Programa de Regularização Ambiental* in Portuguese) aims to restore degraded areas in rural properties, specifically in RLs and APPs areas, that had been illegally converted (Brasil, 12.651/2012). However, this program has not yet been implemented properly by the governments of the South Brazilian states. Only recently some large restoration projects have been initiated, mostly due to specific calls of the Brazilian Biodiversity Fund (*Fundo Brasileiro para a Biodiversidade*, FUNBIO). While this needs to be applauded, limited current knowledge of restoration techniques (see below) may impede full restoration. By implementing the PRA and by reaching other restoration goals, such as those of the PLANAVEG, incentives to create a commercial demand might boost the development of an economic chain of ecological restoration, which is currently incipient in the *Campos Sulinos* region.

20.4 Restoration Strategies and Techniques of *Campos Sulinos* Grasslands

20.4.1 Degradation States and Restoration Strategies

As discussed above, the choice of the restoration strategy and, subsequently, of the most appropriate techniques for restoration will always depend on the degradation state of the site in question (e.g., see Guarino et al. 2023). In general, the more severe degradation, the higher the efforts necessary for restoration, and the longer restoration may take. However, the level of degradation is not always easy to assess, and first impressions may be deceiving. For example, a grassland invaded by alien grasses may at first glance appear closer to a native grassland than a site that until recently has been used as a tree plantation and, after cutting of trees, is devoid of vegetation. However, invasive alien species likely provide an even bigger limitation to restoration than trunk debris and leaf or needle litter layer that challenge grassland restoration after the use with silviculture (Porto et al. 2022). Few studies have been conducted comparing different conservation/degradation states of grassland in the region (Koch et al. 2016, Leidinger et al. 2017). Based on these studies, we can classify degraded sites into two main groups: those where changes in biodiversity and ecosystem properties are related to changes in management regime (i.e., without conversion of grassland vegetation), and those that were affected by land use change, which usually means more severe degradation (Fig. 20.1). However, invasive alien species may become dominant in both situations, which means that case-by-case evaluations are necessary. Fig. 20.1 presents general patterns of changes in abiotic and biotic variables because of different types of degradation (i.e., positive, negative, or neutral effects) observed in *Campos Sulinos* grasslands. The magnitude of these changes can vary due to historic features such as intensity and extent of the degradation process, the grassland ecosystem type, and region, as well as interactions with local environmental conditions. For instance, grasslands in the western portion of RS are more susceptible to degradation by overgrazing than highland grasslands in the northern part of the state because of higher evapotranspiration and more severe drought events in the region.

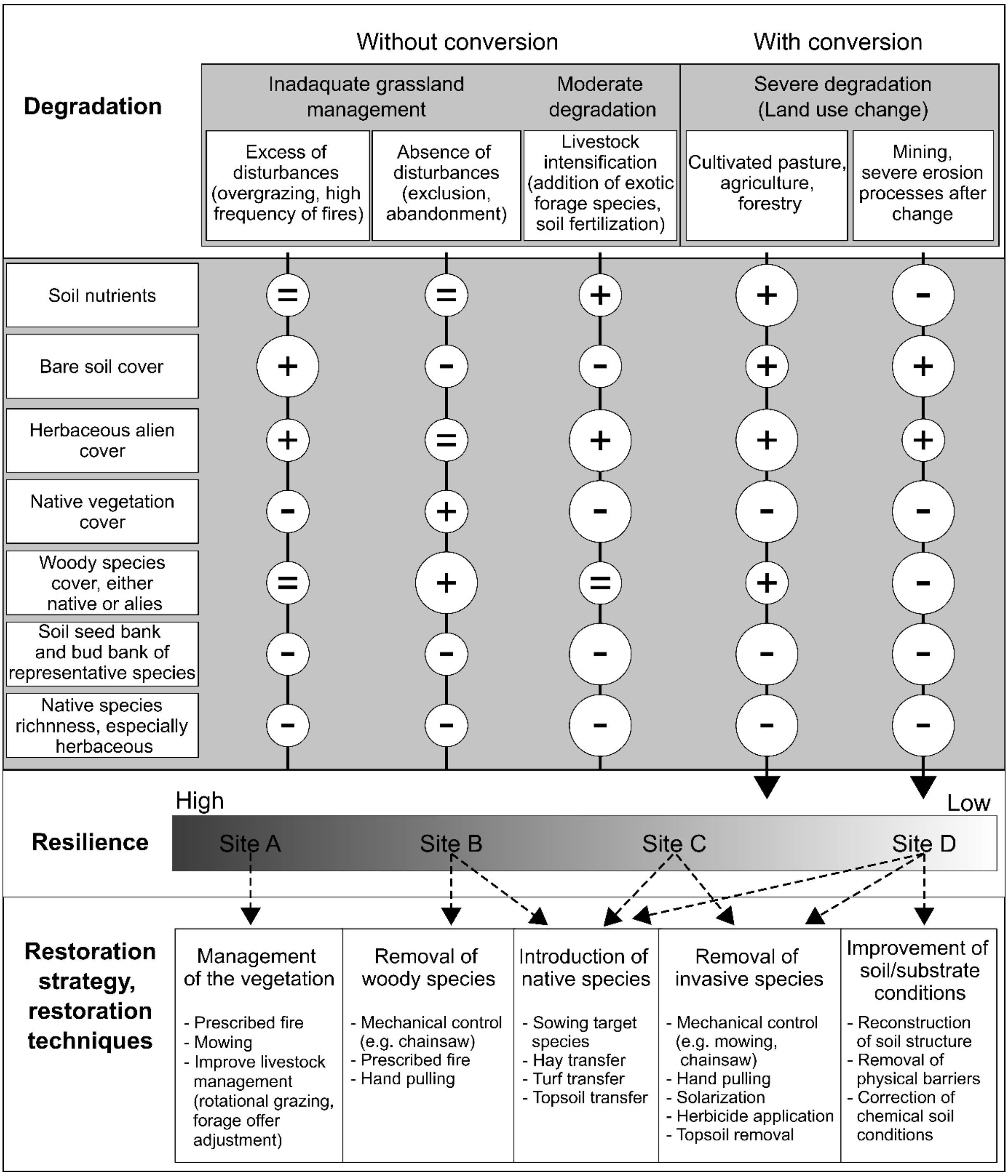

Fig. 20.1 Schematic representation of degradation effects on grassland properties that

further influence ecosystem resilience and subsequent restoration strategies. Symbols (-) and (+) in the circles indicate the general pattern of how degradation influences the abiotic and biotic ecosystem properties. The symbol (=) indicates no or very little effect. The size of the circle indicates the magnitude or strength of the influence. Sources: Baggio et al. 2018, Bonilha et al. 2017, Ferreira et al. 2020, Fidelis et al. 2014, Koch et al. 2016, Leidinger et al. 2017, Pañella et al. 2022, Porto et al. 2022, Silva and Fontana 2020, Sühs et al. 2020, Torchelsen et al. 2019, Vieira et al. 2015, Vieira and Overbeck 2020.

20.4.2 Restoration of Grasslands Degraded by Inadequate Management

When degradation is due to changes in the disturbance regimes (i.e., grazing and fire), abiotic and biotic shifts in the grasslands usually are less intense than at sites with a history of land conversion (Koch et al. 2016, Leidinger et al. 2017). If invasive species are absent, the reintroduction or adjustment of grassland management usually is sufficient to recover typical structure and biodiversity (Fig. 20.2A). Removal of the accumulated biomass and of the woody species is a key step for restoration in these situations; whether this should best be done by fire, grazing, or mowing (Fig. 20.2B, C), or even by a combination of these techniques, depends on the specific situation as well as on the socio-economic context (e.g., availability of domestic grazing animals or mowing machinery). After an initial reduction of biomass, regular management needs to be maintained to achieve grassland restoration targets. However, there may be situations where species introduction is necessary due to the low resilience of most herbaceous species. The diversity of typical grassland species may remain low even after reintroduction of management, as found, for the highland grasslands in RS state, after two decades of abandonment (Thomas et al. 2019a). The reason for this is the commonly low dispersal capacity and the reduction of the bud bank in the soil after long periods without disturbance (Fidelis et al. 2014). The use of fire as a restoration tool could be interesting, however, has not been studied so far in Campos Sulinos grasslands in relation to restoration sites excluded from management (but see Porto et al. 2022); care must be taken to not favor invasive species that respond positively to burns, such as gorse *(Ulex europaeus*; Cordero et al. 2016, Matthews 2005). When degradation is due to overgrazing, the reduction of grazing pressure may already improve the situation. Strategies such as deferred grazing (Fedrigo et al. 2018) or rotational grazing (Boavista et al. 2019) contribute to the fast recovery processes of vegetation. These strategies, easily applicable by landowners, also lead to increased productivity, which is important in the case of privately owned grasslands under grazing (Jaurena et al. 2021).

20.4.3 Restoration of Invaded Grasslands

Invasive species are a major problem throughout the *Campos Sulinos* region (see Guido et al. 2023, in press). Successful cases of true restoration of grasslands degraded by exotic species are still unavailable for the *Campos Sulinos* region, and research has been surprisingly little, given the magnitude of the problem. Studies to control the most problematic invasive species, African lovegrass *E. plana*, are not new (see Coelho 1985). However, historically these studies aimed at controlling the invader to create productive grasslands for livestock raising, using herbicide application and sowing exotic forage species (ex.: Gonzaga and Gonçalves 1999, Perez 2015, Reis et al. 2008), but did not aim at recovery of the native plant community. Despite the advances regarding knowledge of ways to control the invasive species these approaches bring, they cannot be considered to be ecological restoration, because native ecosystems were not a target.

Studies aiming to evaluate techniques to restore native grassland invaded by *E. plana* have been developed from the 2010s on. In lightly invaded areas, adequate grazing management can limit the cover and expansion of *E. plana* (Baggio et al. 2018). Importantly, open soil needs to be avoided, even in lightly invaded areas, as it promotes the establishment and growth of the species (Baggio et al. 2018). In moderately invaded areas, hand-pulling, clipping or herbicide can reduce *E. plana* cover, however, removal of the invader was not sufficient for plant species composition to become similar to that of non-invaded communities (Guido and Pillar 2017). Studies that actively aim to recover plant community composition in grasslands invaded by other species are scarce. Thomas et al. (2019b), working in grasslands dominated by *Urochloa decumbens* (Stapf) R. Webster had an initial success in controlling the invader through chemical (Fig. 20.2G) and topsoil removal (Fig. 20.2F), but did not manage to improve community composition of the grassland. Long-term management actions appear to be necessary to achieve a species composition similar to that of reference grasslands. Chemical control (Fig. 20.2E, F) may not be the best option of choice, because it not always leads to increased similarity of degraded sites with reference sites in terms of community composition (Guido and Pillar 2017). Topsoil removal also is not a useful tool over large areas, due to high costs and the potentially negative effect on native species still in the invaded community. Recently, studies are underway testing the use of plastic tarps to control *U. decumbens*, apparently with some success (L. D. Cezimbra & G.E. Overbeck, unpublished results; see Box 20.1 for more details).

Among woody invaders, gorse (*U. europaeus*) and pine (*Pinus* spp.) are the most problematic species for the grasslands in the region (see Guido et al. 2023, in press). Porto et al. (2022) conducted experiments to restore coastal grasslands degraded by former use for pine plantation. They evaluated different methods to remove the dense layer of pine needles (by controlled burns and mechanical removal) and the efficiency of seed introduction by hay transfer. The removal of the needle layer is a necessary first step in the restoration of grasslands as it impedes the recovery of the vegetation. Due to the higher cost of mechanical removal, controlled burns should be the option that is more feasible for larger areas. Moreover, the application of fire also led to the destruction of pine seeds found in the area: in the mechanical removal treatment, the emergence of pine trees was significantly higher, which presents a considerable obstacle to the restoration of these sites (Porto et al. 2022). This is indicative of fire as a potentially interesting tool to control pine invasion in grasslands.

Independent if invasive species are herbs, shrubs, or trees, in most cases, steps beyond the control and eradication of the invasive species itself are necessary for ecological restoration. The reason is that invasive species generally reduce native plant diversity by their high abundance (e.g., Cezimbra et al. 2021, Guido and Pillar 2017, Thomas et al. 2019b), or due to shading and litter production, as in the case of trees such as pine. Spontaneous recovery of native plant populations often is slow or does not occur at all, and active introduction of plant species is necessary, discussed in the next topic.

20.4.4 Restoration of Sites with a History of Land Conversion

As described above, *Campos Sulinos* grasslands have low resilience to exogenous disturbances. Diversity of native plants usually is considerably reduced in areas under passive restoration after periods of land use with agriculture (old fields) or forestry (Koch et al. 2016, Bonilha et al. 2017, Torchelsen et al. 2020). The plant communities of secondary grasslands differ from their counterparts in primary reference grasslands, often due to the presence of exotic species. Many species that are common in reference grasslands are likely limited in terms of dispersion and establishment success. It has been shown that the soil seed bank even in well-conserved sites does not include the most typical species of the established plant community (Minervini-Silva and Overbeck 2020), which reduces their capacity for natural regeneration by seeds from the soil bank. Natural regeneration from seeds after habitat conversion is even less effective (Vieira et al. 2015, Vieira and Overbeck 2020). For *Campos Sulinos* grasslands, resprouting from belowground bud backs is crucial for the maintenance of plant populations and thus communities crucial (see Fidelis et al. 2014). This means that any process that led to the complete removal of the original grassland vegetation cover is problematic for post-disturbance vegetation recovery. In situations of severe degradation, it is necessary to actively introduce native species. While this still is one of the biggest challenges for grassland ecological restoration in the region, recent studies have contributed to overcome this barrier (Tab. 20.1, Fig. 20.2). In the following, we give an overview of the current state of knowledge of species introduction in *Campos Sulinos* grasslands.

Direct sowing is a well-established technique to restore temperate grasslands in the Northern Hemisphere (Kiehl et al. 2014, Shaw et al. 2020). At current, in the *Campos Sulinos* region, only two native species are sold as seeds (*Axonopus affinis* Chase and *Axonopus compressus* (Sw.) P. Beauv.), primarily for use in gardening (Rolim et al. 2022). Besides these species, seeds of a cultivar (regionally called *pensacola*) of the native *Paspalum notatum* Flüggé, which is highly abundant in grazed grasslands, are available for use as forage species. However, the use of cultivars is generally not recommended for ecological restoration due to risks for genetic diversity (Aubry et al. 2005), failure in successfully achieving a desirable native plant community (Nevill et al. 2016), and seeds not-adapted to harsh local conditions commonly found in degraded sites (e.g., Thomas et al. 2019b). Thomas et al. (2019b) tested direct sowing of 6 g/m^2 of a low diversity mix (three grass species), using cultivated grasses, but establishment success was extremely low. This was likely a consequence of harsh site conditions; possibly native species, better adapted to these conditions, would have performed better. However, seeds of native wild species are inexistent on the restoration market in South Brazil, which constitutes an immense constraint for ecological restoration in the region. Furthermore, the incipient knowledge about the germination and establishment from seeds of native species also complicates attempts to work with seeds collected manually in the field (see Guarino et al. 2018): currently, the risk of failure is high. Clearly, the low availability of native seeds limits the restoration of grasslands in the *Campos Sulinos* region (Dutra-Silva 2023), where, frequently, more than 20 species can be found in one square meter of grasslands, often much more (Menezes et al. 2022).

In response to the lack of seeds on the commercial market, researchers have focused on alternative ways to introduce species in grassland restoration. One interesting approach in this context is the mechanized harvesting of seeds in conserved areas by the use of brush harvesters. Relatively simple machines for this have been previously used in grasslands in Uruguay and Argentina (Machín 2017, Siota et al. 2020). Recently, a similar harvesting equipment was developed in Rio Grande do Sul and is currently under evaluation in pilot projects (Fig. 20.2H). As such machines collect a wide range of mature seeds available in donor grasslands at the moment of harvesting, chances of establishment success are higher: we can expect at least some species to show establishment success at degraded sites.

Another alternative to introduce species is through hay transfer (Fig. 20.2I). This technique aims to introduce species by spreading aboveground biomass that was cut in a well-conserved grassland at a moment when many species present mature seeds (i.e., hay with seeds) over a degraded area. Easy to replicate, low-cost, and successfully employed to restore temperate grasslands in Europe (Kiehl et al. 2010), this technique has been tested in several restoration experiments in the *Campos Sulinos* region (Tab. 20.1). The studies tested single applications of 500 g/m² to 750 g/m² of hay collected once (except for Vieira 2018, who combined two hay harvests). The different studies show a great variation in the success rate in terms of increase plant cover and plant species richness. In the studies of Vieira (2018) and Porto et al. (2022), hay transfer was an efficient technique to introduce native species and increase plant cover in former pine plantations. On the other hand, hay transfer applied by Thomas et al. (2019a, 2019b) did not had the same efficiency in an experimental restoration of an old field invaded by *U. decumbens* and a grassland without management for a long time, respectively. This points out that applicability of the method can vary according to the specific situation where hay is being used or collected. For instance, hay collected in late-summer showed low efficiency in some cases (Thomas et al. 2019b) and good results in others (Vieira 2018, Porto et al. 2022). Specific site conditions, climatic variation, and hay-donor site characteristics may influence the result, just as they should define the ideal amount of hay to be used and when to harvest it. Clearly, results from single studies cannot be easily generalized. Studies at larger scales and under distinct conditions are necessary to better understand the applicability of hay transfer to restore *Campos Sulinos* grasslands.

Independent of how seeds are introduced, it also is important to consider the necessity to improve local site conditions (e.g., Bischoff et al. 2018, Goret et al. 2021, Shaw et al. 2020). Harsh soil conditions (Thomas et al. 2019b) or longer drought periods may lead to failures in the germination and establishment of introduced species.

Improving physical conditions and water availability in the initial restoration phases are actions that obviously require considerable resources, however, may be necessary to avoid failure of restoration. When no elaborate techniques are available, creativity may help: ‘unintentional’ experimentation with tree logs that appear to have both improved microsite conditions and increased seed rain (Fig. 20.2D, see Porto et al. 2023) may serve as inspiration.

Finally, cattle also can be actively used to transport seeds when transferred from sites with large amounts of ripe seeds of target species to degraded sites. Minervini-Silva and Overbeck (2021) showed that large numbers of seeds and species are transported in the feces of domestic cattle, and these seeds can potentially contribute to vegetation development. This alternative should be tested in field conditions; if successful, the use of cattle as a restoration agent could change the game of grassland restoration in private properties, as these grasslands are under livestock grazing which should facilitate restoration.

Table 20.1 Overview of studies that assessed restoration techniques efficiency on vegetation in *Campos Sulinos*.

Objective	**Technique**	**Main results**
Species introduction	Direct sowing	Mix with *Paspalum notatum*, *P. guenoarum,* and *Axonopus affinis* without success (Thomas et al. 2019b)
	Hay transfer	Contrasting results, indicating that the technique has potential, but needs to be further studied (Porto et al., 2022, Thomas et al. 2019a, Thomas et al. 2019b, Vieira 2018)
Invasive grass control	Topsoil removal	Success in controlling *U. decumbens* (Thomas et al. 2019b)
	Herbicide	Success in controlling *U. decumbens* (Thomas et al. 2019b) and *E. plana* (Guido and Pillar 2017)

	Manual removing	Success in controlling *E. plana* with hand-pulling and clipping the aboveground biomass (Guido and Pillar 2017)
Improved site conditions	Prescribed fire	Contrasting results in studies aiming to remove the pine needle layer on the soil and to control pine establishment: positive results in coastal grasslands (Porto et al. 2022), and inefficient in highland grasslands (Vieira 2018)
Vegetation management	Grazing and mowing	Mowing and grazing were efficient to control shrubs and promote plant diversity; but not to control *E. plana* (Dutra-Silva et al. 2022)
	Mowing	Few mowing events (2 or 3) over short periods (less than 9 months) increase plant diversity in a grassland without disturbance for 20 years (Thomas et al. 2019a)
	Deferment and Rotational Grazing	Short temporal exclusions of cattle in overgrazed areas allow the vegetation to recover (Fedrigo et al. 2018). Rotation grazing increased plant biomass and plant diversity (Boavista et al. 2019).
Seed traps	Tree logs	Tree logs placed over bare soil served as seed traps and increased the establishment of wind-dispersed grassland species (Porto et al. 2023)

Fig. 20.2 Different techniques to restore *Campos Sulinos* grasslands that are currently under evaluation: (A) cattle management in degraded areas to restore vegetation structure and diversity; (B) mechanized mowing to remove excess of biomass and (C) manual mowing of abandoned grassland, both to improve light incidence and resprouting; (D) tree logs placed over bare soil are not a restoration technique, but serve as inspiration to develop techniques to increase seed rain and improve site conditions; (E) mechanized chemical control of invasive grasses, such as *Eragrostis plana*; (F) topsoil removal to control invasive grasses; (G) manual herbicide application to control invasive species, such as *Urochloa decumbens*; (H) mechanized seed harvest on donor grasslands to obtain seeds of native species to use for restoration, (I) hay transfer in an experimental plot. Photos: (A) Marcelo Mentges; (B) Rodrigo Dutra-Silva; (C), (D), (F), (H) Pedro Augusto Thomas; (E) Projeto Pró-APA Sustentável; (G) Lua D. Cezimbra; (I) Ana Boeira Porto.

20.5 Current Limitations to Grassland Restoration in the *Campos Sulinos* Region

20.5.1 Species Introduction

The introduction of native species remains a challenge in *Campos Sulinos* grasslands and more studies should be developed. Other techniques then the once mentioned above, for instance transplantation of turfs, topsoil, and seedlings have not yet been tested. We also need to improve our knowledge about germination and establishment of native species, used separately or in mixes. Lists of species that are potentially interesting for use in restoration projects have already been proposed (see Guarino et al. 2018). However, for the majority of species listed, no information regarding strategies for seed collection, optimum storage conditions, germination rate, dormancy breaking requirements, and establishment rate are available, thus limiting their use in real-life restoration beyond experimental plots. Clearly, the lack of seed material is a key constraint for the restoration of grasslands in the *Campos Sulinos* region.

20.5.2 Scale

The majority of the studies listed in Tab. 20.1 were developed as scientific experiments, in small plots or small areas (< 100 m²) and over short periods (mean duration of studies: 2 years). Besides the unquestionable value of such studies, generalizations about the potential of these techniques to contribute to restoration in larger areas should be made with caution. Similarity to the reference system is not rapidly achieved by active restoration in general (Holl and Aide 2011); in the case of the *Campos Sulinos*, the high taxonomic and functional diversity of natural grasslands may turn restoration processes even more time-demanding. A considerable portion of plant species diversity in the *Campos Sulinos* is composed of slow-growing forbs that show a low ability for seedling recruitment and colonization (Overbeck and Müller 2018). Thus, time is an important component to reach ecological restoration success, which will need to be considered in research projects and pilot studies as well. In addition, restoration should always consider the landscape context which can be decisive for the potential of natural recovery (Holl and Aide 2011), but also for the risk of alien species invasions. Experimental plots are limited in this regard.

20.5.3 Fauna

Effectively restored ecosystems obviously consist of more than plants which constitute the component of the ecosystem that often is the main focus of restorationists. So far, consideration of the fauna in the restoration of *Campos Sulinos* grasslands has been very timid, both as restoration target and in monitoring. To our knowledge, only two studies that monitor the effect of restoration, or recovery, of degraded grasslands are available. Bird species richness and abundance showed a rapid recovery in a comparison of secondary grasslands under active restoration with native grasslands (Silva and Fontana 2020). In contrast, ant communities showed lower species diversity and different composition when comparing secondary grassland that recovered without active restoration interventions to primary grasslands under grazing (Dröse et al. 2021).

20.5.4 Social and Economic Aspects of Restoration

There is clear evidence that livestock grazing is compatible with the conservation and restoration of *Campos Sulinos* grasslands. This offers the potential to integrate restoration into productive land management on private properties in a region where cattle ranching is a key economic activity: ecological restoration may improve productivity of grasslands. However, financial incentives are necessary to stimulate restoration, as restoration *per se* is not a priority to landowners (Henderson et al. 2016). At any rate, the development of a restoration market will mean job opportunities and income for human populations and thus improve conditions of life. In Brazil, experiences exist with the creation of networks for the collection of native seeds for ecological restoration in the Cerrado, Amazon, and Atlantic Forest regions (Urzedo et al. 2022). A key element to the success of these experiences is the existence of a demand for seeds from restoration. The implementation of restoration activities, also in response to a legal obligation (e.g., in the context of the PRA; see above), thus appears to be crucial to maintain these networks, or establish new ones, for example in the *Campos Sulinos* region. Restoration of degraded areas in public lands may be a crucial first step towards the development of restoration markets as these areas that are already under the responsibility of public agencies and thus could be restored immediately (Dutra-Silva 2023). Volunteers also may have an important role in restoration and invasive species control programs, especially in small protected areas (see Dechoum et al. 2019). Additionally, projects that stimulate or enhance the participation of local communities in restoration are helpful as they create interest for restoration and illustrate restoration benefits (see Box 20.2). However, at current these potentials are still far from being used to advance restoration.

20.6 Necessary Steps to Advance in Restoration of the *Campos Sulinos*

The sections above clearly indicate that restoration of grasslands in Southern Brazil is at its very beginning. No consolidated techniques to restore grasslands after more severe degradation exist for the region, even though we are well into the UN Decade of Ecosystem Restoration. The inexistence of seeds of native species on the market is a severe limitation for all restoration activities at the moment: Clearly, this is a key issue to be addressed in research, but also by creating the necessary institutional framework, which is still not well developed. In the following, we summarize key issues that need to be addressed to be able to really tackle the ecological restoration of the *Campos Sulinos*.

20.6.1 Raising Awareness and Implementing Priority Actions for Restoration

Advances in ecological restoration do not only depend on knowledge of appropriate techniques and available seeds but, principally, on awareness on the need of restoration and of the important benefits it brings not only to biodiversity but also to society. At current, this awareness still is very low in the *Campos Sulinos* (Porto et al. 2021), which also reflects in slow progress regarding the implementation of restoration activities that are necessary to reach objectives of government strategies, e.g., of Brazil's PLANAVEG. The following actions are necessary:

- Recognition and valorization of the ecological, cultural, and economic importance of the *Campos Sulinos*;
- Implementation of existing governmental programs (such as PRA) for the restoration of grasslands on private land;
- Development of programs to restore degraded areas on public land, mainly in protected areas;
- Development of economic incentives and valorization of products from native grasslands, stimulating conservation and restoration of grasslands;
- Support of land use practices compatible with the biological characteristics of *Campos Sulinos* grasslands, such as traditional grazing.

20.6.2 Advance in Terms of Technical and Scientific Knowledge

All restoration actions should be based on the best scientific information available. Given the lack of knowledge on which restoration activities are the most suitable for specific conditions and especially given the lack of information on seed-based restoration, strengthening research on the field clearly is key to improve restoration. In this context, the following steps are important:

- Development of research activities in different areas of the field of restoration ecology (including restoration techniques, techniques of plant propagation restoration management, restoration monitoring, and social and economic drivers of restoration, among others) to provide conceptual and technical bases for ecological restoration;
- Definition of priority species to be used in restoration, based on clear criteria;

- Development and implementation of monitoring protocols and evaluation of restoration success over a large range of projects;
- Development of reliable cost estimates for grassland restoration in different scenarios;
- Sharing of scientific knowledge on restoration and grassland management beyond academic institutions.

20.6.3 Ecological Restoration in Practice

Restoration in practice needs more than scientific underpinning. In order to work, adequate infrastructure and financing of the restoration sector are necessary.

- Support the development of supply chains relevant to restoration, especially regarding seeds and seedlings;
- Empowerment of local communities, landowners, and stakeholders to become engaged in the restoration process;
- Stimulation of the creation of associations and networks, for example for seed collection;
- Dissemination of successful restoration activities to society.

In the *Campos Sulinos* region, concentrated efforts are necessary to reach the ambitious restoration goals set by PLANAVEG or in international agreements. Even though our knowledge of how to restore grasslands is incipient in many aspects, restoration actions need to be multiplied quickly. This means that researchers, restoration practitioners, and decision-makers need to collaborate to implement ecological restoration and at the same time develop it further. Ecological restoration is key for the future of the *Campos Sulinos*, and this deserves high efforts from society.

Box 20.1: Testing Plastic Tarts to Control an Invasive Grass in a Protected Area

The restoration of areas degraded by invasive grasses still lack efficient protocols, both concerning control of the invasive species and recovery of the native plant community. Protected areas can be considered priority areas for restoration. An interesting example is a project on the control of *Urochloa decumbens* in the Banhado dos Pachecos Wildlife Refuge, Viamão, RS, Brazil. Motivated by the advance of the invasive grass over the habitat of two endangered animal species (the rodent *Ctenomys lami*, and the ground-nesting bird *Hydropsalis anomala*), the objective was to test alternatives techniques to herbicide application to control an invasive grass, using solarization with the help of plastic tarts. The use of tarts for weed control and elimination of other microorganisms is commonly employed in horticulture. In an experimental approach, the effect of black and transparent sheets (Fig. 20.3A) with different lengths of application (30 and 60 days) was evaluated and compared to the effectiveness of herbicide application. Initial results (Fig. 20.3B) show that multiple applications of the plastic sheets are necessary to eliminate individuals of the invasive species that re-establishes from the soil seed bank once the tarps are removed. While a return of native vegetation has been observed in areas adjacent to uninvaded native grasslands, it is still unknown which species are capable of occupying the site once *U. decumbens* is controlled. In the meantime, the park manager has successfully implemented restoration activities over a larger area and with tougher sheets. Future research should monitor vegetation development and, if necessary, develop efficient ways of seed introduction.

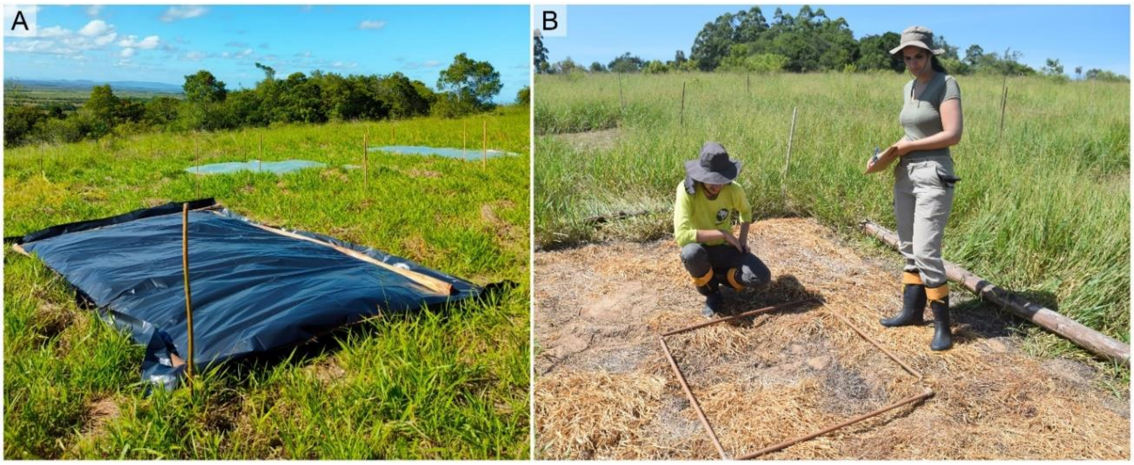

Fig. 20.3 Experiment with plastic tarts to control *Urochloa decumbens* and restore natural grasslands in Banhado dos Pachecos Wildlife Refuge, Viamão, Brazil. (A) Black and transparent plastic tarts (front) and transparent (back); (B) researchers evaluating treatment effects after removal of the plastic sheets. Photos: (A) Lua D. Cezimbra; (B) Luiz Felipe Fonseca da Rocha.

Box 20.2 Integrating Local Communities in Ecological Restoration Projects

The participation of local communities in restoration projects not only increases acceptance but may even lead to the development of economic opportunities. An example is provided by a project on restoration by *Butia* palm groves (Fig. 20.4A) led by the Brazilian Agricultural Research Corporation EMBRAPA. The objectives of the project

'*Rota dos Butiazais: fortalecimento da cadeia produtiva do butiá associada à recuperação da vegetação nativa na região do Parque Estadual do Podocarpus (Encruzilhada do Sul, RS)'* go beyond the restoration of the plant community: It also aims at the of strengthening the productive chain of products derived from the palm *Butia* sp. The project is developed in the region of Podocarpus State Park, in Encruzilhada do Sul, RS, Brazil; a region that has seen strong land use change and where palm groves dominated by *Butia capitata* are typical ecosystems. Alongside active restoration activities, such as transplantation of *Butia* individuals that were at risk and experimentation on grassland restoration, the project also included workshops for the local community (rural producers, local students, teachers, and school cooks) to improve the knowledge of grassland ecology, as well as stimulate interest on the restoration of ecosystems and the sustainable use of the native palm. A culinary workshop, offered to cooks from schools in the region, presented recipes with *Butia* sp., such as cakes and jams, that could gain weight in the local community. A workshop on plant arrangements using native grasses with ornamental potential aimed to change the perspective on natural grassland vegetation in the region by highlighting the potential for the use of native species. Other workshops conducted for the local community presented concepts and techniques of restoration ecology as well as bases for the recognition of native species (Fig. 20.4B). These workshops were important to stimulate the interest of local people in native plants and ecosystems of the region and raised awareness for conservation and maintenance of natural resources.

Fig. 20.4 Photos from workshops conducted within the project in Encruzilhada do Sul, RS, Brazil. (A) *Butia* sp. palm trees associated with grasslands; (B) Workshop presenting the concepts and techniques of restoration ecology. Photos: (A) Rosa Lía Barbieri; (B) Arthur Lenzi da Silva.

Acknowledgments

The authors acknowledge support from the *Conselho Nacional de Desenvolvimento Científico e Tecnológico*, *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Fundação Grupo Boticário* and *FUNBIO* in several projects. We are thankful to the landowners and the protected area managers that permitted us to study ecological restoration in their areas.

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CAPÍTULO 2

The potential of hay transfer for graminoid introduction in the restoration of subtropical grasslands: results from a greenhouse experiment

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Artigo publicado pela revista Flora (308), November, 2023. Doi: 10.1016/j.flora.2023.152398

Title

The potential of hay for graminoid introduction in the restoration of subtropical grasslands: results from a greenhouse experiment

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Abstract

Introduction: Subtropical grasslands have been severely impacted. Due to their low resilience after degradation, active restoration is necessary; however, knowledge still is incipient. Hay transfer is a successfully employed technique to restore other grassland ecosystems and its use is promising in subtropical grasslands. We evaluated the potential of hay transfer for the restoration of the subtropical grasslands in Southern Brazil by assessing the effects of (1) the date of hay harvest and (2) the amount of dry hay used.

Methods: In two protected areas in South Brazilian grasslands, we harvested hay on three dates (mid-Spring, early-Summer, and mid-Summer) and placed it into a greenhouse for seeds in the hay to germinate. Two different amounts of dry hay (375 g/m^2 and 750 g/m^2) were used for each date. We assessed the number of emerging seedlings, species richness, and seedling species composition in each treatment, separately for each site. Emerging forbs were identified as soil seed bank contamination, and only data for graminoids (grasses and sedges) are presented.

Results: The number of seedlings varied among harvest dates, with mid-Spring and early-Summer resulting in more emergencies in both sites. No common pattern to both sites regarding the effect of the amount of dry hay on seedling emergence was found for the two sites, but interaction with the harvest date is important. Species composition and richness also varied across harvest dates, and, independent of the donor site, more hay resulted in more species. Nevertheless, few species were transferred overall.

Conclusions: Hay transfer has the potential to introduce seeds of graminoids and appears to be a promising tool for the restoration of South Brazilian grasslands. There is not a single optimal time for hay harvest nor a pattern regarding hay amount. Few species are established from hay and more than one harvest should be done to increase species richness.

Keywords

Campos Sulinos, hay transfer, restoration ecology, Pampa grasslands, seed germination, seedling emergence

Introduction

Subtropical grasslands are important in terms of biodiversity, economically, and culturally (Buisson et al., 2019; Gibson, 2009). Nevertheless, they have been strongly degraded, mainly due to habitat conversion as a consequence of agriculture, forestry, or mining (Buisson et al., 2019; Millenium Ecosystem Assessment, 2005; Silveira et al., 2020). These ecosystems have low resilience to habitat conversion and secondary grasslands are quite different from primary grasslands (e.g., Fensham et al., 2016; Kirkman et al., 2004; Torchelsen et al., 2019). While the ecological restoration of temperate grasslands in the Northern Hemisphere today is well established and supported by research, many knowledge gaps remain for the restoration of subtropical grasslands, regarding, for example, the potential of seed transferring techniques, the role of fire and herbivory, and how to restore degraded soils (Overbeck and Müller, 2018; Silveira et al., 2020). One important step in ecological restoration is the reintroduction of native species that are absent due to degradation (Buisson et al., 2019). The development of techniques to actively reintroduce species is urgent to achieve ecological restoration targets that benefit the people and conserve biodiversity (Silveira et al., 2020).

Hay transfer is one of the simplest, cheapest, and most effective techniques used to restore Mediterranean (Buisson et al., 2021) and temperate grasslands in Europe (Goret et al., 2021; Kiehl et al., 2010). Hay transfer consists of cutting and collecting the vegetation when seeds of many species are mature but have yet to disperse and then transferring this material to the area to be restored (Buisson et al., 2019). Beyond introducing species, hay can provide safe conditions for seeds to germinate and establish, or act as a mulch layer diminishing erosion, especially when there is bare soil or there are slope conditions (Durbecq et al., 2022; Kiehl et al., 2010). It is important to have a knowledge of which species can be transferred via hay transfer (Wagner et al., 2021). Greenhouse experiments can be a helpful tool for the improvement of knowledge of the potential and limitations of hay transfer (Kiehl et al., 2006; Kirmer and Tischew, 2014; Le Stradic et al., 2014).

The success of hay transfer will depend on the date of hay harvest (e.g., Bischoff et al., 2018). The timing of hay harvest defines the species composition of the seeds to be introduced, which should drive vegetation development and restoration success. To transfer the highest number of typical species, hay should be collected when a large proportion of species is fruiting. However, the effectiveness of different hay collected at different times is hardly evaluated even in well-studied grassland ecosystems (Buisson et al., 2021).

The amount of hay used also influences the seedling establishment and will depend on the plant community and productivity of the donor site, as well as the environmental conditions of the receptor-site (Kiehl et al., 2010). To restore grasslands with low productivity, for example, despite higher seed numbers, the application of thick layers of dry hay (800 g/m²) led to lower relative transfer rates than a thinner layer (Kiehl et al., 2006). Contrary, in mesic grasslands, using a thin hay layer (ratio of 1:3 donor-receptor site) led to a lower number of established species than thicker layers (ratio of 1:1 donor-receptor site; Edwards et al., 2007). To restore temperate European grasslands, hay application can widely range from 180 to 1500 g/m² dry hay (Kiehl et al., 2010) and the effects of differences in hay layer thickness need to be better understood (Valkó et al., 2022).

Subtropical South Brazilian grasslands are species-rich ecosystems (see Andrade et al., 2023) with high economic and cultural importance (Quadros et al., 2015). These grasslands have been severely degraded, and circa 60% of the original cover has already been lost to other land uses (Baeza et al., 2022; Vélez-Martin et al., 2015). Restoration of degraded grasslands is a recent concern in the region and several knowledge gaps remain (Guerra et al., 2020; Overbeck et al., 2013), including knowledge about the ecology of native species that could guide restoration actions. Moreover, seeds of native species are not available commercially for use in restoration projects (Overbeck et al. 2013; Rolim et al. 2022). Thus, the easy application and the low cost of hay transfer make it a promising technique to be used in grassland restoration in the region.

Previous studies in the region tested hay transfer in field experiments and had contrasting results (see Thomas et al., 2019a; Thomas et al., 2019b; Porto et al., 2022), which prevents generalizations about its efficiency, specifically regarding the period of hay harvest and the amounts of hay used. This highlights the need for more studies. The wide range of species dispersing propagules from mid-Spring to mid-Autumn, with species from different functional groups maturing at different times (see Pinheiro et al., 2008; Oleques et al., 2017, and Minervini-Silva et al. under review) make it difficult to define the best date for hay harvest. This study aimed to test the potential of hay transfer for the restoration of South Brazilian grasslands with the help of a greenhouse experiment. The specific objectives were: 1) to assess the effect of different dates of hay harvest and 2) to assess the effect of different amounts of hay used, considering the number of emerged seedlings, species richness, and composition of the seedling community.

Material and Methods

Study sites

Hay was collected in primary grasslands located in two protected areas in Southern Brazil: Lagoa do Peixe National Park and Saint'Hilaire Municipal Natural Park (hereafter, LP and SH, respectively). Both sites are part of Río de la Plata Grasslands, and under a subtropical humid climate (Cfa type, Köppen classification; Peel et al., 2007), without a marked dry period. In both sites, the annual average temperature is 18-19 ºC and the annual rainfall is 1500-1700 mm (Alvares et al., 2013).

LP is located on the coastal plain, on sandy soils. Our hay donor site (WGS84 31° 3'24.90" S, 50° 48'38.52" W; 12 m.a.s.l.) is not managed with fire or cattle, which leads to the accumulation of standing biomass in some places (Fig. 1b). The grasses *Axonopus* aff. *affinis*, *Ischaemum minus*, and *Chascolytrum uniolae* are the most abundant species, and mean richness is 11 species/m² (unpublished data).

SH is located within an urban region and holds mosaics of grasslands and forests, without cattle grazing, but with periodic fires induced by the local population, mainly during Spring and Summer (SH staff, personal communication). At the grasslands of our hay donor site (WGS84 30°6'11.10" S, 51°5'21.76" W; 100 m.a.s.l.) fire is frequent (every two years; Fig. 1a), despite being illegal and unplanned. The grasses *Andropogon* spp., *Aristida* spp., and *Anthaenantia lanata* are the most abundant species, and the mean richness on the sampling unit scale is 29 species/m² (unpublished data).

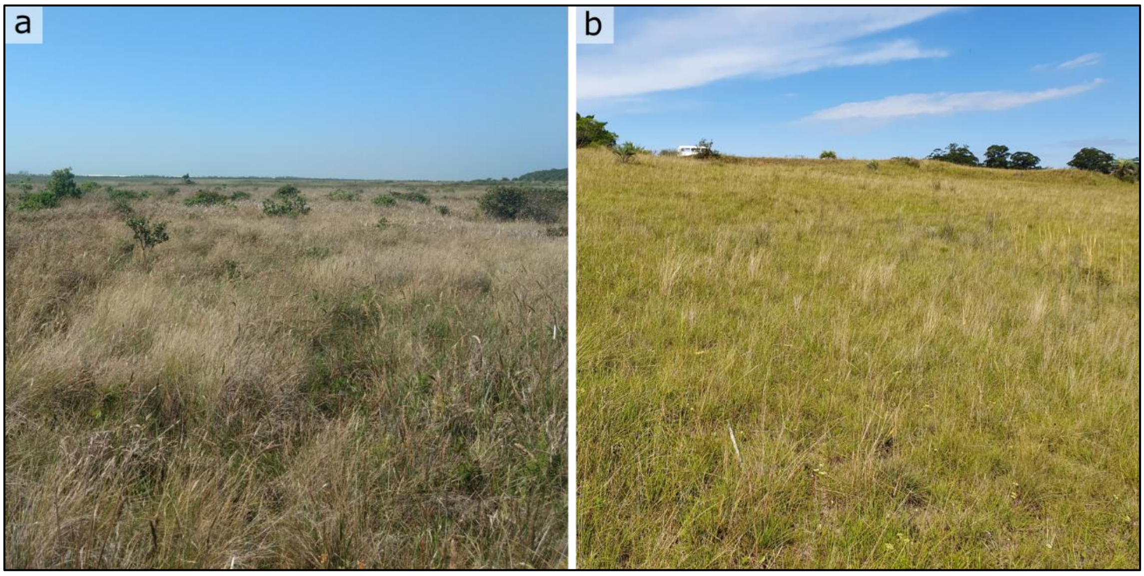

Figure 1. Hay donor sites. (a) Lagoa do Peixe National Park (LP), Mostardas, Brazil; (b) Saint'Hilaire Municipal Natural Park (SH), Viamão, Brazil.

Experimental Design

We established a bi-factorial greenhouse experiment with six treatment combinations. Factor 1 is the date of hay harvest, with three levels: mid-Spring, early-Summer, and mid-Summer. Factor 2 is the amount of dry hay applied in the containers in the greenhouse, with two levels: 375 g/m² and 750 g/m². Previous studies in South Brazilian grasslands applied amounts of dry hay which range from 500 g/m² to 750 g/m² (Thomas et al., 2019a; Thomas et al., 2019b; Porto et al., 2022).

Hay Harvest

At each study site, hay was collected from October 2020 to February 2021 on three different dates (see above the levels of factor 1), during Spring and Summer in the Southern Hemisphere. The interval between each hay harvest date was approximately 45 days (details in Table A.1). We initially had planned to harvest hay a fourth time in early-autumn (late March/early April) to better cover the phenological window of South Brazilian grassland species. However, we had to cancel this fourth harvest due to the Coronavirus pandemic limitations at this time.

At each hay harvest date, we randomly selected three points within each site where a small area of 5 to 8 m² of vegetation was cut with a brush cutter. Areas were representative of the study sites which presented rather homogeneous conditions in terms of vegetation structure and composition. The distances among areas where hay was harvested were, at least, 40 meters at LP, and 150 meters at SH. Before cutting, we registered the species that were dispersing propagules at the study sites (Tables A.2 and A.3). After harvested, in a room with frequent fresh air, hay was spread in shallow plastic trays to avoid mold during the dry process. Once the hay was dry, to each harvest date, the three collections of the same site were homogenized to result in a single bulk sample. Then, small portions of these single bulks were collected, weighted, and separated according to our factor 2 levels to implement the greenhouse experiment. These single bulks were then stored in raffia bags until the start of the greenhouse experiment.

Greenhouse Experiments

We carried out a greenhouse study based on seedling emergence from hay samples. We allocated the amount of dry hay proportional to 750 g/m² and 375 g/m² (the two levels of factor 2) in each container (Fig. A.1). The containers were garden pots (34 cm × 34 cm × 30 cm) filled with sterilized soil and *Carolina Soil®* (a commercial mixture of turf and vermiculite). Control containers with substrate and without hay were used to identify species contamination in the experiment (Fig. A.1). Six replicate containers were set up for the individual treatments, including the controls. The total number of experimental containers was 78: 6 treatments × 6 replicates × 2 study sites + 6 control pots.

All the experimental containers for all the treatments and both study sites were set up on March 9th, 2021. Thus, each hay sample had a different time of storage before the experiment was set up (Table A.1). The containers were watered when necessary, and the frequency of watering varied according to the season. The containers were moved frequently to reduce potential differences inside the greenhouse in terms of luminosity and irrigation. The experiment was carried on at the greenhouse of the Jardim Botânico de Porto Alegre, Brazil. We do not have data about the average temperature inside the greenhouse.

Data collection

After the experiment has been set up, we checked the containers for emerging seedlings counts at intervals of 15 days. Life form (graminoid/forb) was registered for all seedlings which were then identified to the finest taxonomic level possible. Once counted and identified, the seedlings were removed to avoid double-counting. Unidentified seedlings were marked with a toothpick and left in the containers to grow until identification was possible. We monitored seedling emergence for up to 75 days, then we removed the remaining hay from containers and monitored them for more than 70 days, until day 145. At this date, we stopped the monitoring, as no more seedlings emerged. Containers were maintained in the greenhouse until the identification of the remaining unknown species. Species nomenclature follows Andrade et al., (2019).

Data analysis

The two sites were analyzed separately. After the identification of seedlings, we decided to analyze only graminoids (Poaceae and Cyperaceae). Forbs were excluded because the largest part of emerged forb species was considered as experimental contamination (i.e., ruderal species that also appeared in the control containers, and occurred irrespective of the study site or the harvest date; only one individual of Lupinus sp., from LP, was not classified as contamination).

The number of seedling emerging was assessed with Generalized Linear Models (GLMs) using Negative Binomial distribution to deal with overdispersion. The taxon richness of seedlings that emerged was assessed with GLMs using Poisson distribution. All GLMs considered both factors date and amount, and their interaction as predictor variables. Post-hoc tests were performed with emmeans function (emmeans package; Lenth, 2023). Data from seedling species composition from hay samples were assessed with Permutation Multivariate Analysis of Variance (Permanova) considering both factors and their interaction, using Bray-Curtis distance as a resemblance measure, and following pairwise comparison (using pairwiseAdonis function; Arbizu, 2019). Permanova was validated with the Permdisp test (betadisper function from vegan package; Oksanen et al., 2022). To visualize potential differences, an ordination analysis was made by applying a Principal Coordinates Analysis (PCoA) on the Bray-Curtis matrix distance.

All analyses were performed in R (R Core Team, 2023). Other R-packages used were ape (Paradis and Schliep, 2019), car (Fox and Weisberg, 2019), data.table (Dowle and Srinivasan, 2023), ggplot2 (Wickham, 2016), ggrepel (Slowikowski, 2023), and MASS (Venables and Ripley, 2002). The analysis code and data are available on Zenodo (Thomas et al., 2023).

Results

The number of seedlings from the Poaceae and Cyperaceae was variable among both study areas and harvest dates (Fig. 2). In LP, harvest date and the interaction of harvest date and amount of hay, influenced the number of seedlings ($p < 0.0001$ and $p =$

0.0018, respectively; Table A.4), meanwhile, amount of hay did not (Fig. 2a). Mid-Summer had fewer seedlings than mid-Spring and early-Summer, but hay amount interacted with the harvest: less hay resulted in more seedlings in mid-Spring, whereas more amount was better in early-Summer (Table A.6). The most common species was the C3 grass *Chascolytrum uniolae*.

For the hay harvested in SH, both factors and their interaction were significant (p < 0.0001, 0.0128, and < 0.0001, respectively; Table A.7) (Fig. 2b). Here, a higher amount of hay was better, especially in mid-Spring (Tables A.8 and A.10). More seedlings emerged from mid-Spring and early-Summer hays (mainly *Aristida* spp.) than from mid-Summer (Table A.9).

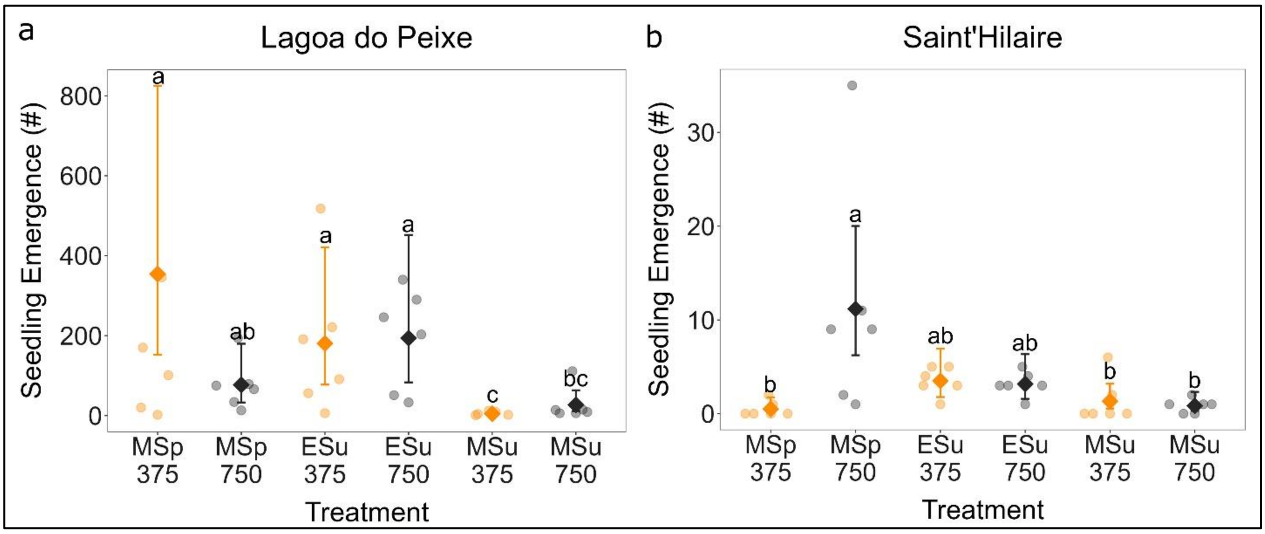

Figure 2. Seedling emergence of graminoids (grasses and sedges) from hay harvested at Lagoa do Peixe (a) and Saint'Hilaire (b), considering three harvest dates and two different hay amounts. Filled diamonds represent predicted values and the vertical bars represent confidence levels. Light circles represent observed values. Treatments codes: harvest date factor: MSp = mid-Spring, ESu = early-Summer, MSu = mid-Summer; dry hay factor: 375 = 375 g/m², 750 = 750 g/m². One outliner value (1486) was removed from the plot at mid-Spring 375 g/m² in Fig.2a, for better visualization. Different lower-case letters indicate significant differences between treatments. Note differences in the scale of the y-axis.

Species richness of seedlings was influenced only by the amount of hay in LP (Fig. 3, Tables A.11 and A.12), while in SH both the harvest date and the amount of hay had an effect (Fig. 3, Tables A.13, A.14 and A.15). The interaction of factors was not significant.

In LP containers, we identified 16 species (including seven morphospecies that could not be fully identified). The most abundant species was *Chascolytrum uniolae* (4698 seedlings), followed by Cyperaceae 1 and *Ischaemum minus* (105 and 62 seedlings, respectively). In SH containers, we identified 14 species (including six morphospecies); the most abundant taxa were *Aristida* spp. and *Chascolytrum lamarckianum*, with 51 and 36 seedlings, respectively. For *Aristida* spp., only a part of the seedlings could be identified to the species level, thus seedlings from this genus were considered as a group (full lists of emerged species are in Tables A.16 and A.17).

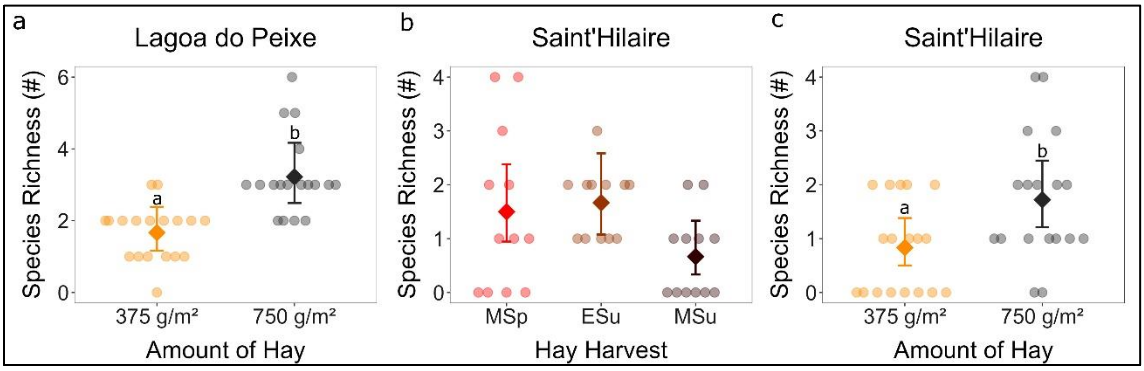

Figure 3. Species richness of seedlings emerged on hay harvested at Lagoa do Peixe (a) and Saint'Hilaire (b – c), according to the date of hay harvest (b) and amount of dry hay (a – c). Filled diamonds represent predicted values and the vertical bars represent confidence levels. Light circles represent observed values. Treatments codes to date of hay harvest factor: MSp = mid-Spring, ESu = early-Summer, MSu = mid-Summer. Different lower-case letters mean different significance between treatments.

Regarding the graminoid community composition at the two study sites, only harvest date had an effect ($p = 0.0001$ at LP (Table A.18), and 0.0005 at SH (Table A.19)). The following pairwise comparison showed differences among the three dates at LP (Table A.20), while at SH, mid-Summer and early-Summer dates differed from each other, and both did not differ from mid-Spring (Table A.21). In the plot diagram for LP, there is a clear separation of mid-Spring and early-Summer samples from mid-Summer samples along the first axis, and a distinction between mid-Spring and early-Summer along the second axis (Fig. 4a). For SH, samples from the three dates are more intermingled, but most early-Summer samples are in the right portion of the diagram (Fig. 4b). However, as both ordination analyses showed heterogeneous sample dispersion across groups (Permdisp analysis), seedling species composition differences between treatments should be interpreted with caution.

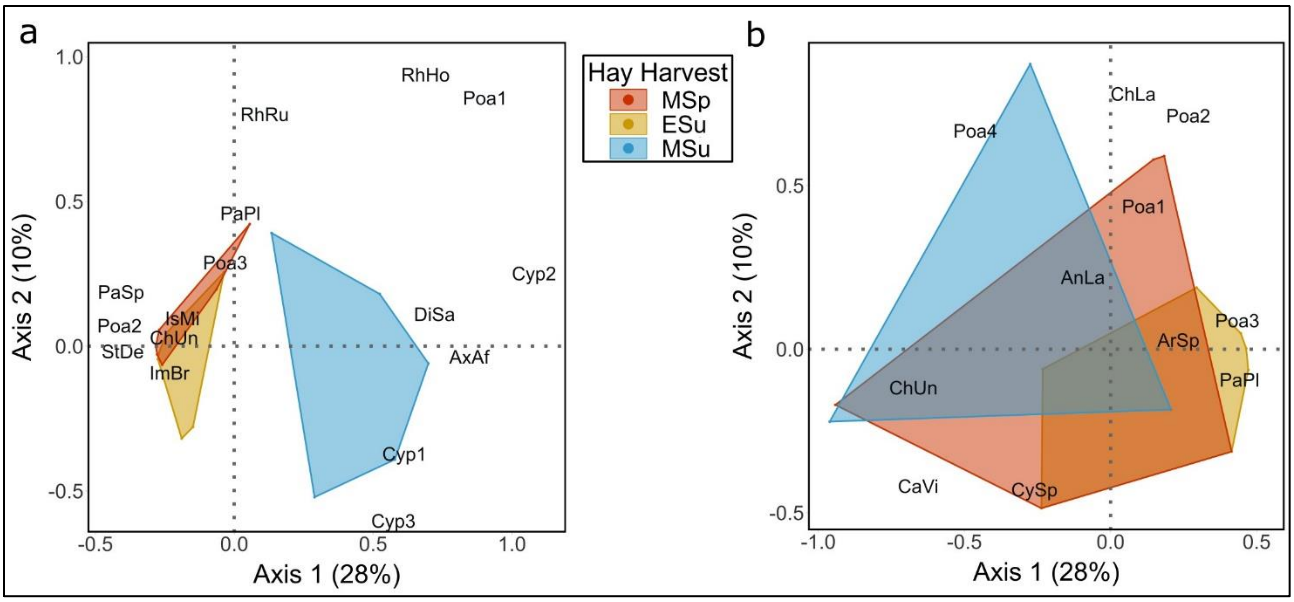

Figure 4. Ordination diagram of the Principal Coordinates Analysis (PCoA) based on emerged grass and sedge species and Bray-Curtis distance as similarity measure for the second experiment in Lagoa do Peixe (a) and Saint'Hilaire (b). MSp = mid-Spring, ESu = early-Summer, MSu = mid-Summer. (a) AxAf, *Axonopus* aff. *affinis*; ChUn, *Chascolytrum uniolae*; Cyp1, Cyperaceae 1; Cyp2, Cyperaceae 2; Cyp3, Cyperaceae 3; DiSa, *Dichantelium sabulorum*; ImBr, *Imperata brasiliensis*; IsMi, *Ischaemum minus*; PaSp, *Paspalum sp*.; PaPl, *Paspalum plicatulum* Michx.; Poa1, Poaceae 1; Poa2, Poaceae 2; Poa3, Poaceae 3; RhHo, *Rhynchospora holochoesnoides*; RhRu, *Rhynchospora rugosa*; StDe, *Steinchisma decipens*. (b) AnLa, *Anthaenantia lanata*; ArSp, *Aristida* spp.; CaVi, *Calamagrostis viridiflavecens*; ChLa, *Chascolytrum lamarckianum*; ChUn, *Chascolytrum uniolae*; CySp, *Cyperus sp*.; PaPl, *Paspalum plicatulum*; Poa1, Poaceae 1; Poa2, Poaceae 2; Poa3, Poaceae 3; Poa4, Poaceae 4.

Discussion

Our results from a greenhouse study indicate that the introduction of hay is a potentially interesting technique for reintroducing graminoid species in the restoration of subtropical grasslands. In previous studies in the region, where hay did not increase native species richness or plant cover, it was not possible to identify the reason for the lack of success of the technique, which could be due to the absence of seeds in the hay of the donor site or be consequence of unfavorable microsite conditions at the degraded receptor site (Thomas et al., 2019a; Thomas et al., 2019b). Our study suggests that the lack of viable seeds in hay is not the main limitation for grasses and some sedges, at least for grasslands like those of LP.

There was a great variation between sites and dates on seedling emergence, not only in terms of quantity of seedlings but also of species composition. Our study indicates that mid-Spring and early-Summer were the best dates for hay harvesting when focusing on the total quantity of seedlings. At SH, during these periods, different species of *Aristida*, locally very abundant (e.g., *A. flaccida, A. laevis, A. jubata*), disperse seeds: these species that generally present high germination rates (see Overbeck et al., 2006b; Guido et al., 2017) contributed considerably to seedling emergence. Mid-Summer showed less potential for hay harvest at both sites. On the other hand, in a field experiment, also conducted in LP, which tested hay that was collected in mid-Summer had good results (Porto et al., 2022). The reason for this divergence may be that the mid-Summer in 2021, when we collected hay, was unexpectedly dry, which may have negatively impacted propagule production. It is important to bear in mind that seed production, even in periods identified as generally favorable, will always be affected by variations in climate conditions (Werner et al., 2020). This highlights the importance of frequently monitoring plant phenology at hay donor site (e.g., every 15 days), especially of target species, to identify the best moment for hay harvest. As South Brazilian grasslands are characterized by high species richness (plot and site scale) and high beta diversity (e.g., Overbeck et al., 2006a; Menezes et al., 2016), with species composition greatly differing among sites, optimal timing for hay harvest may also differ across sites and regions.

Regarding hay amounts, there was not consistent pattern among sites. Higher amounts of hay resulted in more seedlings' emergence for SH, meanwhile, no differences were detected for LP. We interpret these differences as being related to the vegetation characteristics of each donor site in consequence of the frequency of biomass removal by fire. In southern Brazil, grassland species are adapted to fire and/or grazing) (Andrade et al., 2019; Behling et al., 2007; Overbeck et al., 2018), and those grasslands that have been excluded from fires for a long time (as LP) have large amounts of accumulated plant biomass, in contrast to sites with regular burnings (as SH) (Goldas et al., 2022). Longer time since fire also changes the leaf trait values on the grassland vegetation at the community level, increasing LDMC and decreasing SLA (Abedi et al., 2022), which is related to a slower leaf decomposition (Vaieretti et al., 2005; Pérez-Harguidenguy et al., 2013). Therefore, at SH, where donor grasslands burn frequently (every 2 years), there is a low accumulation of fibrous and standing dead biomass (Fig. A.2), and even a thick hay layer (750 g/m²) decomposes rapidly and should not act as a barrier to seedling emergence. In contrast, the donor site at LP did not have recurrent fires (nor grazing) that remove aboveground biomass; in consequence, the vegetation is composed of more fibrous tussock grasses and has a higher proportion of standing dead biomass (mostly *Chascolytrum uniolae* and *Imperata brasiliensis* Trin.; Fig. A.3), and that decomposes more slowly, limiting seedling emergence (e.g., Pilon et al., 2018). Consequently, when harvesting hay from grasslands without disturbances (i.e., fire and grazing), in regions where productivity is high, the use of a thin hay layer is better than a thicker one for the emergence of seedlings, even if more seeds might be present in higher hay amounts.

Regarding species richness, our results showed that more hay led to the emergence of more species. However, the overall number of species that emerged from hay was very low, especially considering the high plant richness in SH vegetation (Tables A.2 and A.16). A sizeable proportion of species from donor site usually fail to establish from hay (Wagner et al., 2021). At donor sites where several species occur with generally lower abundance, such as SH, there is a higher risk of failure to capture their seeds in the hay or of failure in germination requirements that might prevent immediate establishment after hay transfer (Wagner et al., 2021). Contrary, at the LP donor grassland, vegetation is less diverse, and few species are highly abundant (e.g., *Axonopus* aff. *affinis*, *Ischaemun minus*, and *Chascolytrum uniolae*); their seeds appear to dominate the community in the hay and had good germination capacity. Indeed, one single species, *Chascolytrum uniolae,* was responsible for more than 90% of emerged seedlings at LP. Abundant species whose seeds can be captured by hay harvest at donor sites should, in theory, be established readily after the transfer (Wagner et al., 2021). These same three species were successfully introduced by hay collected in LP in a field experiment (Porto et al. 2022). This indicates the potential of introducing, by hay, species such as these, with have high germination and establishment rates. At the same field experiment, the species richness transferred by hay was higher than in our experiment even three months after hay transfer (mean 5.3 species/m² using 750 g/m² fresh hay), when combined with litter removal (mean five species). It is also likely that certain species failed to germinate in the greenhouse when compared with field experiments because their specific germination requirements, such as temperature fluctuations, that are better supplied in the field (Stevenson et al., 1997).

The seedling community from LP hay formed two groups of graminoid species, one with mid-Spring and early-Summer hay samples and the other with mid-Summer samples. As we do not find differences due to harvest date for species richness, it seems interesting to collect hay, at least, at two moments (i.e., in mid-Spring or early-Summer, plus in mid-Summer), to achieve higher species richness in the community established from seeds in transferred hay. Combining hay from different dates is another promising way to increase the efficiency of hay transfer (Bischoff et al., 2018; Valkó et al., 2022). Regarding SH, the low efficiency of mid-Summer hay for species richness and seedling emergence and the fact that seedling community composition did not show clear differences through the dates of hay harvest indicate that mid-Spring and early-Summer dates should be preferred instead of mid-Summer harvest. However, as discussed above, local phenological monitoring can better inform the timing for harvesting hay to achieve two relevant restoration aims – introducing target species and species diversity.

The emergence of forbs (which are less abundant in our study grasslands, despite high species richness) was so low that we did not even analyze the forbs data. In South Brazilian grasslands, fire increases abundance and richness of flowering forbs over short time scales (one to six months), and decreases over time (Fidelis and Blanco, 2014; Goldas et al., 2022). We observe few forbs fruiting at the time of hay harvest (Tables A.2 and A.3). To use prescribed fires some months before hay harvest could increase the efficiency of hay transfer for the introduction of forb (and grass) species, but this should be tested.

Differences in species composition between hay donor and receptor sites are common in grassland restoration, mainly when assessed over short periods (Bischoff et al., 2018; Mudrák et al., 2018; Valkó et al., 2022; Wagner et al., 2021). Additional actions should be taken to improve restoration results of diversity and composition indicators, such as combining hay from multiple donor sites (Valkó et al., 2022), having additional sowings (Mudrák et al., 2018; Török et al., 2012; Valkó et al., 2022; Wagner et al., 2021), or using freshly cut green hay (Kiehl et al., 2010) and a proper soil preparation (Bischoff et al., 2018; Jaunatre et al., 2014; Porto et al., 2022). In future studies, the use of fresh hay should be preferred instead of dry hay due to lower loss of seeds when handling the hay (Kiehl et al., 2010). In our study, we could not calculate the rate of success of species transfer by hay from a donor site to a recipient site (see Kiehl et al., 2010), as we did not realize a detailed vegetation sampling before hay harvest and were not able to identify all emerged seedlings from hay. We encourage future studies to do that to further understand the efficiency of species transfer by hay in the process of restoration.

Greenhouse studies are important to determine seed content in hay but must be carefully interpreted as species can differ from each other in their germination requirements (Wagner et al., 2021). We here discussed potential barriers to seedling emergence when using a high amount of hay, but under field conditions decomposition of hay is likely to be faster than in a greenhouse and higher amounts of hay might be advantageous because of the higher quantity of seeds and species they may contain. Field experiments with a longer evaluation time also will be helpful in registering the establishment of species with seed dormancy, especially to Poaceae and Cyperaceae families that present a large number of species with physiological dormancy (Baskin and Baskin, 2014; Kildisheva et al., 2020). For example, Porto et al., (2022) found persistent positive effects of hay transfer on species richness and vegetation cover after 24 months. Most studies of hay transfer available are from small-scale experiments (Kiehl et al., 2010), including this and previous studies in South Brazilian subtropical grasslands. Future studies and restoration projects should increase experimental scale and test potential factors that influence the success of using hay in field conditions, including the effect of micro-sites, seed predation, and climatic variation, which may be limiting factors for plant establishment. Importantly, as indicated through the variation among sites and the interaction that we found among both factors, it is still too early to generalize about the best way to use hay transfer in the restoration of subtropical grasslands.

Conclusion

Our experiment shows that hay transfer has the potential to introduce graminoid seeds when restoring the South Brazilian grasslands. Nevertheless, the variation in the number of seedlings regarding the site and date of harvest is in accordance with the contrasting results in previous studies using hay in our region. This highlights how species introduction can be a hard task in grassland restoration and calls for continuous development of this technique. In addition, the absence of forbs emerging from hay highlights the need to employ other techniques to ensure the introduction of this life form. Grassland management (fire and/or grazing) at the hay donor sites should be considered when planning hay harvesting. We hope that these findings be helpful in the restoration of other grasslands, mainly subtropical ecosystems.

Acknowledgments

We thank all our colleagues that helped with fieldwork, plant identifications, experiment setup and maintenance, and data analysis. We also thank the staff at Lagoa do Peixe National Park, Saint'Hilaire Municipal Natural Park, and Jardim Botânico of Porto Alegre.

Data availability

Data on seedling emergence (number, species richness, and community) from hay in the greenhouse experiment and the R-scripts to analyze them are available at Zenodo (Thomas et al., 2023). Data of vegetation sampling in the hay-donor site are available under request.

Funding

This work was supported by Bolsas Funbio – Conservando o Futuro (grant from Funbio and Instituto Humanize to PAT), by CNPq (grant 432356/2018-5 to GEO) and Fundação Boticário (grant 1132_20182 to GEO). GEO is supported by CNPq (310345/2018-9). This study is partially financed by CAPES (Finance Code 001).

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Supplementary Material

Table A.1. Dates of each hay harvest at Lagoa do Peixe and Saint'Hilaire.

Study Site	Hay Harvest Date Level Factor	Date	Time of Hay Storage Until the Experiment Set Up (March 9th, 2021)
Lagoa do Peixe	mid-Spring	18 November 2020	111 days
	early-Summer	22 December 2020	77 days
	mid-Summer	10 February 2021	27 days
Saint'Hilaire	mid-Spring	12 November 2020	117 days
	early-Summer	21 December 2020	78 days
	mid-Summer	9 February 2021	28 days

Table A.2. Floristic list of species dispersing propagules in hay donor sites in Saint'Hilaire, Viamão, Brazil, per dates of hay harvest. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

Family	Species \ Date Harvest Factor	MSp	ESu	MSu
Apiaceae	*Eryngium ciliatum* Cham. & Schltdl.		x	x
	Eryngium sanguisorba Cham. & Schltdl.	x	x	x
Asteraceae	*Chromolaena ascendens* (Sch.Bip. ex Baker)			x
	Chromolaena hirsuta (Hook. & Arn.) R.M.King & H.Rob.		x	
	Chromolaena squarrulosa (Hook. & Arn.) R.M.King & H.Rob.			x
	Chrysolaena flexuosa (Sims) H.Rob.			x
	Schlechtendalia luzulifolia Less.	x		
Campanulaceae	*Wahlenbergia linarioides* (Lam.) A.DC.			x
Cyperaceae	*Bulbostylis capillaris* (L.) C.B.Clarke			x
	Bulbostylis sphaerocephala (Boeckeler) C.B.Clarke	x		
	Fimbristylis dichotoma (L.) Vahl	x		
	Rhynchospora barrosiana Guagl.	x	x	x
	Rhynchospora setigera (Kunth) Griseb.	x		
Fabaceae	*Chamaecrista repens* (Vogel) H.S.Irwin & Barneby		x	
	Desmanthus tatuhyensis Hoehne	x		x
	Macroptilium prostratum(Benth.) Urb.			x
	Nanogalactia heterophylla (Gillies ex Hook. & Arn.) L.P.Queiroz	x		
Iridaceae	*Sisyrinchium vaginatum* Spreng.	x	x	
Linaceae	*Cliococca selaginoides* (Lam.) C.M. Rogers & Mildner	x		
Orobanchaceae	*Buchnera cf. longifolia*			x
Poaceae	*Andropogon leucostachyus* Kunth			x
	Andropogon selloanus (Hack.) Hack.	x	x	x
	Aristida filifolia (Arechav.) Herter	x		
	Aristida flaccida Trin. & Rupr.	x	x	

	Aristida jubata (Arechav.) Herter	x		
	Aristida laevis (Nees) Kunth	x	x	
	Axonopus suffultus (Mikan ex Trin.) Parodi	x		x
	Chascolytrum lamarckianum (Nees) Matthei	x		
	Chascolytrum subaristatum (Lam.) Desv.	x	x	
	Chascolytrum uniolae (Nees) L.Essi, Longhi-Wagner & Souza-Chies	x		
	Danthonia cirrata Hack. & Arechav.		x	
	Elionurus muticus (Spreng.) Kuntze	x	x	x
	Nassella melanosperma (J.Presl) Barkworth	x		
	Panicum olyroides Kunth	x	x	x
	Panicum peladoense Henrard	x	x	x
	Paspalum plicatulum Michx.	x		x
	Piptochaetium montevidense (Spreng.) Parodi Hack. Ex Arechav.		x	x
	Piptochaetium stipoides (Trin. & Rupr.)	x		
	Schizachyrium microstachyum (Desv. ex Ham.) Roseng., B.R.Arrill. & Izag.			x
	Trachypogon spicatus (L.f.) Kuntze	x		
Rubiaceae	*Borreria capitata* (Ruiz & Pav.) DC.		x	
	Galianthe fastigiata Griseb.			x
	Richardia grandiflora (Cham. & Schltdl.) Steud.	x	x	x

Table A.3. Floristic list of species dispersing propagules in hay donor sites in Lagoa do Peixe, per dates of hay harvest. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

Family	Species \ Date Harvest Factor	MSp	ESu	MSu
Apocynaceae	*Oxypetalum* sp.			x
Araliaceae	*Hydrocotyle bonariensis* Lam.			x
Cyperaceae	*Eleocharis contracta* Maury ex Micheli			x
	Fuirena robusta Kunth			x
	Rhynchospora barrosiana Guagl.		x	
	Rhynchospora brittonii Gale			x
	Rhynchospora holoschoenoides (Rich.) Herter		x	x
	Rhynchospora rugosa (Vahl) Gale			x
	Rhynchospora tenuis Link	x	x	x
	Scleria distans Poir.	x	x	x
Fabaceae	*Desmodium adscendens* (Sw.) DC.			x
Juncaceae	*Juncus* cf. *microcephalus*			x
Lentibulariaceae	*Utricularia tricolor* A.St.-Hil.		x	
Melastomataceae	*Chaetogastra versicolor* (Lindl.) P.J.F.Guim. & Michelang.			x
Poaceae	*Axonopus* aff. *affinis* Chase			x
	Chascolytrum uniolae (Nees) L.Essi, Longhi-Wagner & Souza-Chies	x	x	
	Danthonia secundiflora J.Presl	x	x	

Dichanthelium sabulorum (Lam.) Gould & C.A. Clark		x	x
Imperata brasiliensis Trin.	x	x	
Ischaemum minus J.Presl	x	x	x
Panicum aquaticum Poir.			x
Paspalum pumilum Nees			x
Schizachyrium cf. *plumigerum*			x
Schizachyrium condensatum (Kunth) Nees		x	x
Schizachyrium sp.			x

Table A.4. Analysis of Deviance Table for the number of seedlings to LP hay.

	Df	Deviance	Resid. Df.	Resid. Dev.	Pr(>Chi)
NULL			35	84.713	
date	2	30.7907	33	53.922	< 0.0001
amount	1	0.0187	32	53.903	0.8913
date:amount	2	12.5358	30	41.367	0.0018

Table A.5. *Post-hoc* pairwise comparison (emmeans function) on the interaction between factors (date*amount) to number of seedlings to LP hay. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer. 375g = 375 g/m²; 750g = 750 g/m².

Contrast	estimate	SE	df	Z ratio	*p* value
ESu-375g × MSu-375g	3.6553	0.639	Inf	5.723	<.0001
ESu-375g × MSp-375g	-0.674	0.61	Inf	-1.104	0.8799
ESu-375g × ESu-750g	-0.0713	0.611	Inf	-0.117	1
ESu-375g × MSu-750g	1.9061	0.615	Inf	3.099	0.0239
ESu-375g × MSp-750g	0.8541	0.612	Inf	1.396	0.7296
MSu-375g × MSp-375g	-4.3293	0.638	Inf	-6.782	<.0001
MSu-375g × ESu-750g	-3.7266	0.639	Inf	-5.835	<.0001
MSu-375g × MSu-750g	-1.7492	0.643	Inf	-2.721	0.0711
MSu-375g × MSp-750g	-2.8012	0.64	Inf	-4.379	0.0002
MSp-375g × ESu-750g	0.6028	0.61	Inf	0.987	0.9221
MSp-375g × MSu-750g	2.5801	0.615	Inf	4.197	0.0004
MSp-375g × MSp-750g	1.5281	0.611	Inf	2.499	0.1243
ESu-750g × MSu-750g	1.9774	0.615	Inf	3.215	0.0165
ESu-750g × MSp-750g	0.9254	0.612	Inf	1.513	0.6562
MSu-750g × MSp-750g	-1.052	0.616	Inf	-1.707	0.5268

Table A.6. *Post-hoc* pairwise comparison (emmeans function) between harvest date levels on number of seedlings to LP hay. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

contrast	estimate	SE	df	Z ratio	*p* value
ESu × MSu	2.816	0.443	Inf	6.352	<.0001

ESu × MSp	0.126	0.432	Inf	0.291	0.9545
MSu × MSp	-2.691	0.444	Inf	-6.066	<.0001

Table A.7. Analysis of Deviance Table on number of seedlings to SH hay.

	Df	Deviance	Resid. Df.	Resid. Dev.	Pr(>Chi)
NULL	35	82.323			
date	2	18.625	33	63.697	< 0.0001
amount	1	6.1941	32	57.503	0.0128
date:amount	2	20.670	30	36.833	< 0.0001

Table A.8. *Post-hoc* pairwise comparison (emmeans function) on the interaction between factors (date*amount) on number of seedlings to SH hay. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer. 375g = 375 g/m²; 750g = 750 g/m².

contrast	estimate	SE	df	Z ratio	*p* value
ESu-375g × MSu-375g	0.965	0.566	Inf	1.706	0.5278
ESu-375g × MSp-375g	1.946	0.727	Inf	2.677	0.0798
ESu-375g × ESu-750g	0.1	0.498	Inf	0.201	1
ESu-375g × MSu-750g	1.435	0.629	Inf	2.283	0.2008
ESu-375g × MSp-750g	-1.16	0.458	Inf	-2.532	0.1149
MSu-375g × MSp-375g	0.981	0.778	Inf	1.26	0.8067
MSu-375g × ESu-750g	-0.865	0.57	Inf	-1.517	0.6532
MSu-375g × MSu-750g	0.47	0.687	Inf	0.684	0.9838
MSu-375g × MSp-750g	-2.125	0.536	Inf	-3.965	0.0010
MSp-375g × ESu-750g	-1.846	0.73	Inf	-2.527	0.1161
MSp-375g × MSu-750g	-0.511	0.825	Inf	-0.619	0.9897
MSp-375g × MSp-750g	-3.106	0.704	Inf	-4.412	0.0001
ESu-750g × MSu-750g	1.335	0.633	Inf	2.111	0.2816
ESu-750g × MSp-750g	-1.26	0.464	Inf	-2.718	0.0717
MSu-750g × MSp-750g	-2.595	0.602	Inf	-4.311	0.0002

Table A.9. *Post-hoc* pairwise comparison (emmeans function) between harvest date levels on number of seedlings to SH hay. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

contrast	estimate	SE	df	Z ratio	*p* value
ESu × MSu	1.15	0.424	Inf	2.71	0.0184
ESu × MSp	0.343	0.431	Inf	0.795	0.7059
MSu × MSp	-0.807	0.492	Inf	-1.641	0.2285

Table A.10. *Post-hoc* pairwise comparison (emmeans function) between amount of hay levels on number of seedlings to SH hay. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

contrast	estimate	SE	df	Z ratio	*p* value
375g × 750g	-0.845	0.368	Inf	-2.3	0.0215

Table A.11. Analysis of Deviance for species richness of seedlings to LP hay.

	Df	Deviance	Resid. Df.	Resid. Dev.	Pr(>Chi)
NULL	35	22.612			
date	2	1.6151	33	20.997	0.4459
amount	1	9.0659	32	11.931	0.0026
date:amount	2	0.0107	30	11.92	0.9946

Table A.12. *Post-hoc* pairwise comparison (emmeans function) between amount of hay levels on species richness of seedlings to LP hay. 375g = 375 g/m²; 750g = 750 g/m².

contrast	estimate	SE	df	z.ratio	p.value
375g × 750g	-0.66	0.227	Inf	-2.908	0.0036

Table A.13. Analysis of Deviance for species richness of seedlings to SH hay.

	Df	Deviance	Resid. Df.	Resid. Dev.	Pr(>Chi)
NULL	35	40.539			
date	2	5.9911	33	34.548	0.0500
amount	1	5.6832	32	28.864	0.0171
date:amount	2	3.8553	30	25.009	0.1459

Table A.14. *Post-hoc* pairwise comparison (emmeans function) to the interaction between factors (date*amount) on species richness of seedlings on SH hay. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

contrast	estimate	SE	df	Z ratio	*p* value
ESu × MSu	0.896	0.421	Inf	2.129	0.0841
ESu × MSp	0.379	0.39	Inf	0.971	0.5950
MSu × MSp	-0.517	0.474	Inf	-1.09	0.5203

Table A.15 *Post-hoc* pairwise comparison (emmeans function) between amount of hay levels on number of seedlings data to SH. 375g = 375 g/m²; 750g = 750 g/m².

contrast	estimate	SE	df	Z ratio	*p* value
375g × 750g	-0.672	0.351	Inf	-1.914	0.0556

Table A.16. List of seedling species from hay harvested in Saint'Hilaire, per dates of hay harvest. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

Species \ Date Harvest Factor	MSp	ESu	MSu	Total
Anthaenantia lanata (Kunth) Benth.	7	-	-	7
Aristida sp.	17	29	1	47

Aristida filifolia (Arechav.) Herter	-	1	-	1
Aristida flaccida Trin. & Rupr.	1	-	-	1
Aristida jubata (Arechav.) Herter	2	-	-	2
Calamagrostis viridiflavescens (Poir.) Steud. (Poir.) Steud.	5	4	2	11
Chascolytrum lamarckianum (Nees) Matthei	34	-	2	36
Chascolytrum uniolae (Nees) L. Essi, Longhi-Wagner & Souza-Chies	-	-	6	6
Cyperus sp.	-	1	-	1
Paspalum plicatulum Mich×.	-	2	-	2
Poaceae 1	-	2	1	3
Poaceae 2	4	-	-	4
Poaceae 3	-	1	-	1
Poaceae 4	-	-	1	1
Total	70	40	13	123

Table A.17. List of seedling species from hay harvested in Lagoa do Peixe, per date of hay harvesting. MSp = mid-Spring; ESu = early-Summer; MSu = mid-Summer.

Species \ Date Harvest Factor	MSp	ESu	MSu	Total
Axonopus aff *affinis* Vahls (ined.)	-	-	38	38
Chascolytrum uniolae (Nees) L. Essi, Longhi-Wagner & Souza-Chies	2554	2132	11	4697
Cyperaceae 1	-	-	105	105
Cyperaceae 2	-	-	1	1
Cyperaceae 3	-	-	6	6
Dichanthelium sabulorum (Lam.) Gould & C.A. Clark	-	1	5	6
Imperata brasiliensis Trin.	26	16	-	42
Ischaemum minus J.Presl	3	58	1	62
Paspalum sp.	-	4	-	4
Paspalum plicatulum Michx.	-	10	2	12
Poaceae 1	-	-	1	1
Poaceae 2	1	-	-	1
Poaceae 3	2	-	1	3
Rhynchospora holoschoenoides (Rich.) Herter	-	-	6	6
Rhynchospora rugosa (Vahl) Gale	-	19	12	31
Steinchisma decipiens (Nees ex Trin.) W.V. Br.	-	6	-	6
Total	2586	2246	189	5021

A.18. Permanova results for the community of seedlings emerged from LP hay.

	Df	SumOfSqs	R²	F	Pr(>F)
date	2	4.048	0.27972	6.224	0.0001
amount	1	0.4029	0.02784	1.2388	0.1970
date:amount	2	0.5901	0.04078	0.9073	0.5617

residual	29	9.4305	0.65166		
total	34	14.4714	1		

A.19 Permanova results for the community of seedlings emerged from SH hay.

	Df	SumOfSqs	R²	F	Pr(>F)
date	2	2.1765	0.14934	2.0884	0.0005
amount	1	0.6832	0.04688	1.3111	0.1341
date:amount	2	1.2927	0.0887	1.2404	0.1345
residual	20	10.4219	0.71509		
total	25	14.5742	1		

Table A.20. Results of pairwise.Adonis (Arbizu 2020) as *posthoc* comparison to community of seedling species to dates of the hay harvest to LP.

	Df	SumsOfSqs	F.Model	R²	*p* value	*p* adjusted
MSp × MEu	1	0.301	3.744	0.145454	0.0038	0.0038
MSp × MSu	1	2.633	14.775	0.413008	0.0001	0.0001
ESu × MSu	1	2.655	15.378	0.422736	0.0001	0.0001

Table A.21. Results of pairwise.Adonis (Arbizu 2020) as *posthoc* comparison to community of seedling species to dates of the hay harvest to SH.

Contrast	Df	SumsOfSqs	F.Model	R²	*p* value	*p* adjusted
MSp × MEu	1	0.3193	2.112	0.105	0.0983	0.0983
MSp × MSu	1	0.666	1.986	0.142	0.0862	0.0983
ESu × MSu	1	1.342	6.563	0.290	0.0004	0.0012

Figure A.1. Experimental pots with dry hay (left and middle) and control pot without hay (right).

Figure A.2. Measuring the height vegetation in Saint'Hilaire Municipal Natural Park, Viamão, Brazil. Note the low quantity of dead plant biomass accumulated.

Figure A.3. Cutting the grassland with a brush cutter to collect hay in hay donor sites at the Lagoa do Peixe National Park, Mostardas, Brazil. Note the vegetation height and standing dead biomass present.

CAPÍTULO 3

Seed density and limiting similarity to reduce invasive grass performance in sown communities for grassland restoration purposes

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Artigo a ser submetido no periódico Applied Vegetation Science, como Research Article.

Title

Seed density and limiting similarity to reduce invasive grass performance in sown communities for grassland restoration purposes

Running Title

Higher seed density is a better approach

Authors

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Abstract

- Questions: Invasive species are one of the major threats to grassland ecosystems, and seed addition is a key step in their ecological restoration. Nevertheless, to define seed mixes and seed quantities is a hard task. To design seed mixes using functional traits and create a community based on limiting similarity to outcompete the invader is an interesting approach. We aimed to test the effect of two sowed communities, one created by limiting similarity, and three seed density sowing to reduce the performance of the invasive grass Eragrostis plana.
- Location: Campos Sulinos grasslands, Southern Brazil.
- Methods: Using nine native grasses we created a bifactorial experiment. The first factor was the sowed community: (1) Limiting, based on limiting similarity, and (2) Balanced, where all species have the same proportion at the final seed density. We used the leaf functional traits SLA, LA, and LDMC to create the Limiting community to the invasive species. The second factor was the seed density sowing of native species: (1) 1 g/m^2, (2)

2 g/m², and (3) 4 g/m², which were sowed twice. Eragrostis plana was sowed once with 0.5 g/m². The experiment was carried out in a greenhouse for nine months. Aboveground and belowground biomass of all species were collected at the end of the experiment to compare the treatment effects over the invasive performance.
- Results: Biomass production of invasive species was lower when sowed with native species, no matter the sowed community. Higher seed density of native species resulted in lower invasive aboveground biomass, but not belowground biomass. Some native species did not germinate, consequently, the established communities were quite different from the seed mixes designed.
- Conclusions: Eragrostis plana is a strong competitor and increasing the seed density sowing is a better approach when defining sowing strategies to compete with invasive grasses.

Keywords

Campos Sulinos, ecological restoration, *Eragrostis plana*, Invasive species, Leaf functional traits, Limiting similarity, Seed density, Subtropical grasslands

Introduction

Invasive species are one of the major threats to grassland ecosystem functioning and biodiversity (IPBES, 2018) and one of the major challenges in ecological restoration projects (Buisson et al., 2019; Funk et al., 2008; Meyerson & D’Antonio, 2002). Their control has been considered a priority issue in grassland restoration (Buisson et al., 2021; Kaul & Wilsey, 2021; Török et al., 2021). Additionally, to restore functional and diverse ecosystems, it is fundamental to reintroduce native species that are absent or present with low cover due to degradation (Buisson et al., 2019; Hedberg & Kotowski, 2010) so that a native plant community develops after, or concomitantly with, control of exotic species.

Seed addition is the most cost-effective alternative for restoration (Pedrini et al., 2020) and the most common method to introduce species in different grassland ecosystems (Buisson et al., 2021; Hedberg & Kotowski, 2010; Kiehl et al., 2010; Laurance et al., 2010; Palma & Laurance, 2015). However, further research is needed on which native seed mixes and species combinations best prevent the invasion of alien species (Kaul & Wilsey, 2023). One option that has been suggested is designing seed mixes in grassland restoration in a way that the future community better compete with invasive species, according to the principle of limiting similarity (Funk et al., 2008; Yannelli et al., 2018).

The limiting similarity theory argues that species with similar functional traits limit each other due to competition for the same resources (MacArthur & Levins, 1967; Funk et al., 2008). Therefore, by creating a native community similar to an invader, we expect that the invasive species will have lower performance once its niche is overlapped by those of the native species (Hooper & Dukes, 2010; Hulvey & Aigner, 2014). We can use a functional trait approach to design these seed mixes (Funk et al., 2008; Laughlin, 2014). Functional traits are species characteristics (morphological, physiological, and phenological) that are associated with their ecological strategies and how they respond to environmental factors (Pérez-Harguindeguy et al., 2013; Violle et al., 2007) or affect ecosystem functioning (Díaz et al., 2007). Leaf traits have been found to represent the main functional strategies of the economic spectrum in plants. Specific Leaf area (SLA) is a proxy for rapid resource acquisition and growth, where species with high-SLA values are relatively fast-growing and good light competitors but tend also to be highly palatable to herbivores and have lower longevity (Mathakutha et al., 2019; Ordonez et al., 2010; Pérez-Harguindeguy et al., 2013). Leaf area (LA) is indicative of photosynthetic area and ability to deal with environmental stresses, such as nutrient stress or drought, and herbivory; a small LA value is considered a conservative state related to resource limitation, plant defense, slow growth, and drought and cold tolerance (Mathakutha et al., 2019; Pérez-Harguindeguy et al., 2013). Leaf dry matter content (LDMC) is related to resistance to physical hazards, such as herbivory, wind, and frost: higher values mean more resistance, but also slower growth (Pérez-Harguindeguy et al., 2013). Together, these traits may affect plant performance of species assemblages under establishment for the restoration of functional ecosystems.

Seed density is another major factor that can influence native species establishment success in restoration projects (e.g., Walker et al., 2015; Barr et al., 2017). On the one hand, sowing high-density mixtures of seeds has been shown to reduce the abundance of exotic species in grasslands (Barr et al., 2017; Carter & Blair, 2012), and to be more important than limiting similarity in the seed mix (Yannelli et al., 2017, 2018) by resulting in more native cover and native biomass production. On the other hand, other studies have shown that high-density mixes have no effect on vegetation development (Gillhaussen et al., 2014; Scotton, 2019), i.e., cannot control invasive species (Nemec et al., 2013). In this context, the order of arrival (Gillhaussen et al., 2014), seed source and growth form (Walker et al., 2015), and to increase seed mix diversity (Barr et al., 2017; Nemec et al., 2013) can be more important than seed density to achieve different restoration targets. The question of seed density is also important from an economic point of view: high-density mixes can cost twice more than low-density mixes (Török et al., 2011), which may be a constraint in practice. The definition of an optimal sowing density is required to avoid wasting seed material (Török et al., 2011; Walker et al., 2015), especially in a regional context where native seeds are not available commercially.

In Southern Brazil, the Campos Sulinos grasslands are subtropical grasslands of high biodiversity (e.g., Overbeck et al., 2022) that can be considered old-growth grasslands (sensu Veldman et al., 2015). In the southern part of the Campos Sulinos, the tussock grass *Eragrostis plana*, originally from southern Africa, is the most problematic invasive species (Guido & Guadagnin, 2015; Guido et al., 2016). Because of its fibrous leaves, cattle avoid it, which gives it a competitive advantage and allows it to outcompete native species, resulting in species-poor grasslands and economic losses in cattle production (Medeiros et al., 2009; Dresseno et al., 2018; Guido & Guadagnin, 2015). The species has great potential to spread and invade other grasslands, as in Uruguay and Argentina (Barbosa et al., 2013). Studies to control *E. plana* are not new (e.g., Coelho, 1985), however, a major part of studies focus on herbicide application (Coelho, 1985; Gonzaga & Gonçalves, 1999; Goulart et al., 2009; Reis et al., 2008). So far, only one study sowed species to outcompete *E. plana*, using exotic forage and native species (Medeiros & Ferreira, 2011), with positive results primarily from the introduction of exotic species combined with soil disturbance. The selection of native species that can effectively compete with *E. plana* is fundamental for the future of the Campos Sulinos, to restore invaded grasslands or degraded areas from different land uses, as the invader propagule pressure is incredibly high and widespread along the roads.

We aimed to test the potential of sowing native communities to reduce the performance (aboveground and belowground biomass) of invasive *E. plana* in a greenhouse experiment. The first objective was to compare the effect of two different native communities composed of the same species but with different species abundances to compete with the invader: one community designed to overlap the invader niche, based on leaf functional traits weighted by species relative abundance, and the second community with all species with the same initial relative abundance. The second objective was to test the effect of different seed densities of native species on the invader performance; for this, we used the two communities above in three different seed densities. We hypothesized that the native community designed to overlap the invader would be more efficient in reducing its performance because of limiting similarity and that this effect would be stronger at higher seed densities due to stronger competition for the invasive species.

Methods

Sown Native Species

We used nine native grasses: *Anthaenantia lanata, Aristida jubata, Aristida laevis, Axonopus affinis, Bromus catharticus, Chascolytrum uniolae, Chascolytrum subaristatum, Paspalum notatum, Paspalum plicatulum* (Table 1). These species were selected according to characteristics that make them interesting for restoration projects in the Campos Sulinos region, such as abundance, biomass production in native grasslands, and, mainly, the ease to collect seeds. In addition, we had previous data on the viability of seed germination of these species (Table S1). While our species number is rather low, especially when compared to the plant diversity of Campos Sulinos grasslands (Andrade et al., 2023), the commercial availability of native seeds from the Campos Sulinos region is extremely low (Rolim et al., 2022), which means that restorationists need to focus on species that are easy to collect (Barak et al., 2022). The species *A. affinis, B. catharticus, C. subaristatum, P. notatum*, and *P. plicatulum* have been previously cited as priority species for restoration (Guarino et al., 2018). *P. plicatulum* has already been cited as a potential species that can outcompete *E. plana* (Medeiros & Ferreira, 2011). Species name followed the Flora do Brasil (Brazil Flora Group, 2021).

Table 1. Data on seedling emergence, life form, and photosynthetic pathway of the Poaceae species used in our competition experiment. The proportion of each native species in each experimental designed seed mix (limiting and balanced). Emergence data source: *Anthaenantia lanata, Aristida jubata, Aristida laevis, Paspalum plicatulum,* and *Eragrostis plana* from Roitman (2021); *Axonopus affinis* from Isla Sementes; *Paspalum notatum* from Colonial Sementes, and, in bold, from our tests (Supp. Mat. 1). Full information about origin of seeds and data emergence sources on Table S1.

Species	Emergence	Life Form	C3/C4	Limiting (%)	Balanced (%)
Anthaenantia lanata	77	Tussock grass	C4	34	11

Aristida jubata	76	Tussock grass	C4	7	11
Aristida laevis	90	Tussock grass	C4	5	11
Axonopus affinis	96	Prostate grass	C4	7	11
Bromus catharticus	**92**	Tussock grass	C3	4	11
Chascolytrum subaristatum	**98**	Tussock grass	C3	7	11
Chascolytrum uniolae	**94**	Tussock grass	C3	8	11
Paspalum notatum	70	Prostate grass	C4	5	11
Paspalum plicatulum	67	Tussock grass	C4	23	11
Eragrostis plana	68	Tussock grass	C4	-	-

Leaf Functional Traits

We used data from three leaf functional traits: specific leaf area (SLA), leaf area (LA), and leaf dry matter content (LDMC). Functional traits data used were those measured by Silva et al. (2020) and Hoss et al. (under review), and available at the Levcamp Trait Data Bank (unpublished data). For *B. catharticus* and *C. uniolae*, for which no data are available, we measured traits according to the protocols in Pérez-Harguindeguy et al. (2013). The leaf traits values and their sources are presented in Table S2.

Definition of the Plant Community based on the principle of Limiting Similarity

The Laughlin model (2014) returns, for a given species pool, the species relative abundance to achieve a functional target in a species assemblage. In our case, the objective was a plant community whose functional trait spaces overlap that of the invader. The model suggests the proportion of each species (from our given pool – Table 1), based on their functional traits, to achieve a maximum similarity to our functional target, that’s it, the invader functional trait space. For that, the model uses systems of linear equations and quantitative traits. Systems of linear equations are useful for estimating unknown probabilities, given a set of equality and inequality constraints (Yannelli et al., 2018). We used the FD package (Laliberté et al., 2014) to model species proportion at the seed mix to overlap the trait space of the invader *E. plana*. To contrast with this community based on the principle of limiting similarity, another community with the same species but without variance in the relative proportion between the species (i.e., a Balanced community – Table 1) was established.

Experimental Design

We created a bi-factorial experiment. The first factor is “Community”, with two distinct seed mixes: (1) seed mix with limiting similarity based on the model proposed by Laughlin 2014 (hereafter Limiting community), and (2) seed mix with equal proportions of all native species (hereafter Balanced community). The proportion of each species in each seed mix (Limiting and Balanced communities) is presented in Table 1. The species present in both communities are the same nine species, and the communities differ only in the relative abundance of each species based on seed weight. We do not expect differences in native plant biomass production between both designed seed mixes, because they were assembled to evaluate the effects on the invader biomass, but we expected that higher densities should result in higher biomass.

The second factor is the native seed density sowing (“Density”), with three levels of total seed weight that was sown in each sample of the community: (1) 1 g/m², (2) 2 g/m², and (3) 4 g/m². We sowed these quantities twice (see below at Greenhouse Experiment and Data Collection). The use of seed weights instead of seed density means that small-seeded species were sown at higher densities than large-seeded ones. There is no protocol that defines the best seed densities to be used for restoration ecology in Campos Sulinos grasslands. Similar experiments to restore semi-natural grasslands in Germany used 3 g/m² (Möhrle et al., 2021; Rojas-Botero et al., 2022; Yannelli et al., 2018), and 1.5, 2.5 and 5 g/m² (Gillhaussen et al., 2014). Experiments in Central Europe grasslands generally present good results with 1–5 g/m² (Kiehl et al., 2010). We used the Density factor to define the seed quantities in each treatment. For example, in the Limiting community, *A. lanata* should be present at 34%. At 1 g/m² density, 0.34 g of *A. lanata* were sowed/m². Values of seed dried weight per species are presented in Table S3. The number of added seeds and percentage over the total number of added native seeds based on seed dry weight (Table S3) to each treatment are presented in Table S4.

In all treatments, the invasive species were sowed once at 0.5 g/m². This value simulates the density of *E. plana* in the soil seed bank at heavily degraded sites. Previous studies had identified a seed bank density of invaded areas present with 963 to 2040 seeds/m² (Ferreira et al., 2008; Reis et al., 2008). As the fresh weight of a thousand seeds of *E. plana* is 0.23 g (Focht & Medeiros, 2012), this translates into a soil seed bank from 0.22 g/m² to 0.47 g/m². The application of 0.5 g/m² of *E. plana* seeds thus seeks to reproduce the soil seed bank of a highly invaded area. We had also a monoculture of *E.*

plana, as a control treatment, where only the invasive species was sowed, to compare the invasive performance to the experimental treatments. All treatments had five replicates.

Seeds

Seeds were collected in different areas in southern Brazil in 2020 and 2021 (more details in Table S1) and then stored in paper bags in dry ambient conditions until the experiment was set up. Emergence data of *A. lanata, A. jubata, A. laevis*, *P. plicatulum*, and *E. plana* were obtained from a previous experiment performed by Roitman (2021) using the same batch of collected seeds that we used. For the other three species, *B. catharticus*, *C. subaristatum*, and *C. uniolae*, we performed emergence tests, detailed in Supplementary Material 1. We used purchased seeds of *A. affinis* and *P. notatum*. These species are very abundant in Campos Sulinos grasslands, mainly in grazed areas. Seeds of *A. affinis* are sold for landscaping purposes, while *P. notatum,* specifically the 'Pensacola' cultivar, is intended for forage use.

Greenhouse Experiment and Data Collection

We set up the greenhouse experiment on March 4th, 2022, late summer in the Southern Hemisphere. In garden pots (34 × 34 × 30 cm) filled with sterilized soil and *Carolina Soil*® (a commercial mixture of turf and vermiculite), we sowed all the native species and the invasive at the same time, and applied a fine layer of substrate to cover the seeds. Irrigation was done with sprinkles, when necessary, usually 2-3 times/week, depending on the plant growth stages and the temperature inside the greenhouse. Pots were distributed by chance and were frequently moved inside the greenhouse to diminish possible differences in luminosity and irrigation. At the end of August, as we did not observe the germination and establishment of all native species, we decided to apply a second sowing of native species, repeating the quantities used at the first time. Before that, between August 29 and 31, 2022, we cut all the aboveground biomass (vegetation height approx. 5 cm) for each species in all pots so that the new seeds would have light and space to germinate and establish. Then, on September 1st, 2022, we applied the second sowing. Thus, the final quantities of added native seeds were 2 g/m^2, 4 g/m^2, and 8 g/m^2. After the sowing, we applied another fine layer of substrate to cover the seeds.

Between December 5 and 13, nine months after the first sowing, we harvested the biomass in all experimental plots. We collected aboveground and belowground biomass for each species in each pot. Collected biomass was stored in paper bags and oven-dried for, at least, 48 hours at 70°C, and then, immediately weighed.

Data Analysis

At the end of the experiment, the established communities in the pots did not properly represent the designed seed mixes. Of the nine native species sowed, only six germinated and established: *A. jubata, A. laevis, B. catharticus*, *C. subaristatum*, *C. uniolae*, and *P. plicatulum*. The species *A. lanata, A. affinis*, and *P. notatum* were not present in the established community. Individuals of *A. jubata* and *A. laevis*, and of *C. subaristatum* and *C. uniolae* were too small to enable a proper identification to the species level for all individuals, thus we grouped them inside the same genus and presented the results using *Aristida* spp. and *Chascolytrum* spp.

The effects of both factors and their interaction on *E. plana* biomass and on total native biomass were analyzed through Analysis of Variance (ANOVA). As we had an incomplete design, we did not consider Control pots as a factor in these tests, but we showed the results to visually see the invader's performance when it was alone in the pots. ANOVA assumptions were checked using a performance package (Lüdecke et al., 2021). Post-hoc comparisons were performed with a Tukey test.

The species composition at the community level of established plants was analyzed contrasting both factors and their interaction, using Permutation Multivariate Analysis of Variance (PERMANOVA). Community data were first transformed using Hellinger and then the Bray-Curtis dissimilarity was applied. Permanova assumptions were checked using the Permdisp test to assess group centroids' homoscedasticity (betadisper function from vegan package; Oksanen et al., 2022). When significant, PERMANOVAs were followed by post-hoc comparisons made with pairwise-Adonis (Arbizu, 2019). To visualize potential differences at the community level, non-metric multidimensional scaling (NMDS) ordination analyses were run on the same matrix distance.

All analyses were performed in R. Data and analysis codes are available at Zenodo Repository (Thomas et al., 2023).

Results

Effect of Factors on Invasive Species Biomass

E. plana biomass was higher than native plant biomass regardless of the sowing treatments and factors (Community and Density). *E. plana* biomass did not differ within the Community factor, neither for aboveground nor belowground biomass (Fig. 1A; Table S5). For the Density factor, differences were observed only on the aboveground biomass ($F = 4.155$, $p = 0.028$): higher density of native seeds resulted in lower *E. plana* biomass (Fig. 1B; Table S6). The interaction between factors was not significant, both for aboveground and belowground biomass. Although not formally tested, *E. plana* biomass was higher in the control treatment than in treatments with native species (Fig. 1A - B).

Effect of Factors on Native Species Biomass

The Limiting community produced more native plant belowground biomass than the Balanced community ($F = 5.921$, $p = 0.022$; Fig. 1C; Table S5). Contrary, there was no significant effect of the Community factor on the aboveground biomass of native plants. The Density factor had significant effects on both aboveground and belowground biomass of native plants ($F = 5.710$, $p = 0.009$, and $F = 5.966$, $p = 0.007$, respectively; Table S5). The 4 g/m^2 treatment produced higher amounts than 1 g/m^2, and both treatments did not differ from 2 g/m^2 treatment, which produced intermediate amounts of aboveground and belowground biomass (Fig. 1D; Tables S7 and S8). The interaction between factors was not significant. After analyzing the species individually, we saw that *B. catharticus* contributed to most of the biomass of the Balanced community, while P. *plicatulum* contributed most to the Limiting community (Table S9). These species were sown with 11% and 23% of total seed weight at each treatment, respectively (Table 1).

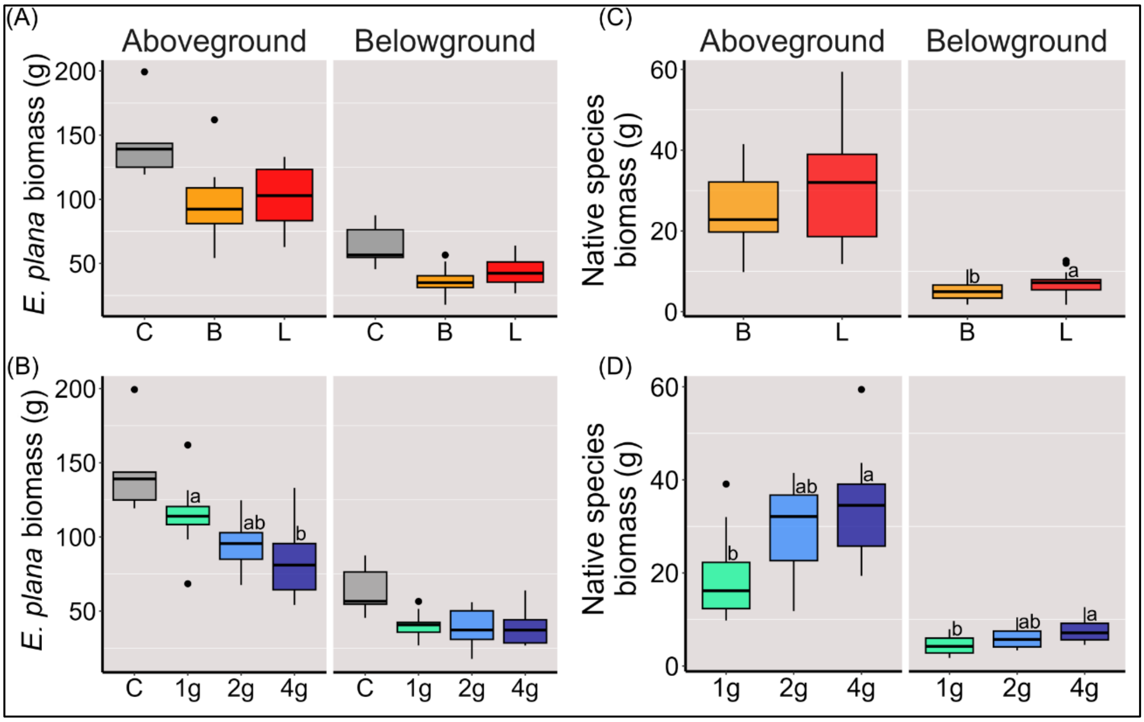

Figure 1. *Eragrostis plana* aboveground and belowground biomass (g) according to Community factor (A) and Density factor (B). Total native species aboveground and belowground biomass according to Community factor (C) and Density factor (D). Treatment codes: C = Control, B = Balanced, L = Limiting, 1g = 1 g/m², 2g = 2 g/m², and 4g = 4 g/m². Different letters denote significant differences between treatments. The control plots were not included in the ANOVAs and are shown here as a reference of the invader performance.

Effect of Factors of Community Composition

Both factors (Community and Density) had a significant effect on species composition described by the relative aboveground biomass of the species in the communities (F = 7.213, $p < 0.001$, and F = 1.8861, p = 0.019, respectively; Table S10). Balanced communities had more *Bromus* spp. and *Aristida* spp., meanwhile Limiting communities had more *P. plicatulum* and *Chascolytrum* spp. *E. plana* was positioned near of the ordination plot center, but more related to Balanced communities, i.e., their biomass was relatively higher in these communities compared to the co-occurring species present in the Limiting communities. Regarding to the Density factor, there is a separation in species composition only between the 1 g/m² treatment, with more *E. plana*, and 4 g/m², with more *P. plicatulum* and *Bromus* spp. (F = 1.863, p = 0.019; Fig. 2B; Table S10).

For belowground biomass, only the Community factor had a significant effect (F = 4.966, $p < 0.001$; Table S10). Again, the pattern in the NMDS is the same as that for aboveground biomass, where Balanced communities had more *E. plana, Bromus* spp., and *Aristida* spp., meanwhile Limiting communities had more *P. plicatulum* and *Chascolytrum* spp. Interactions between factors were not significant for both aboveground and belowground biomass.

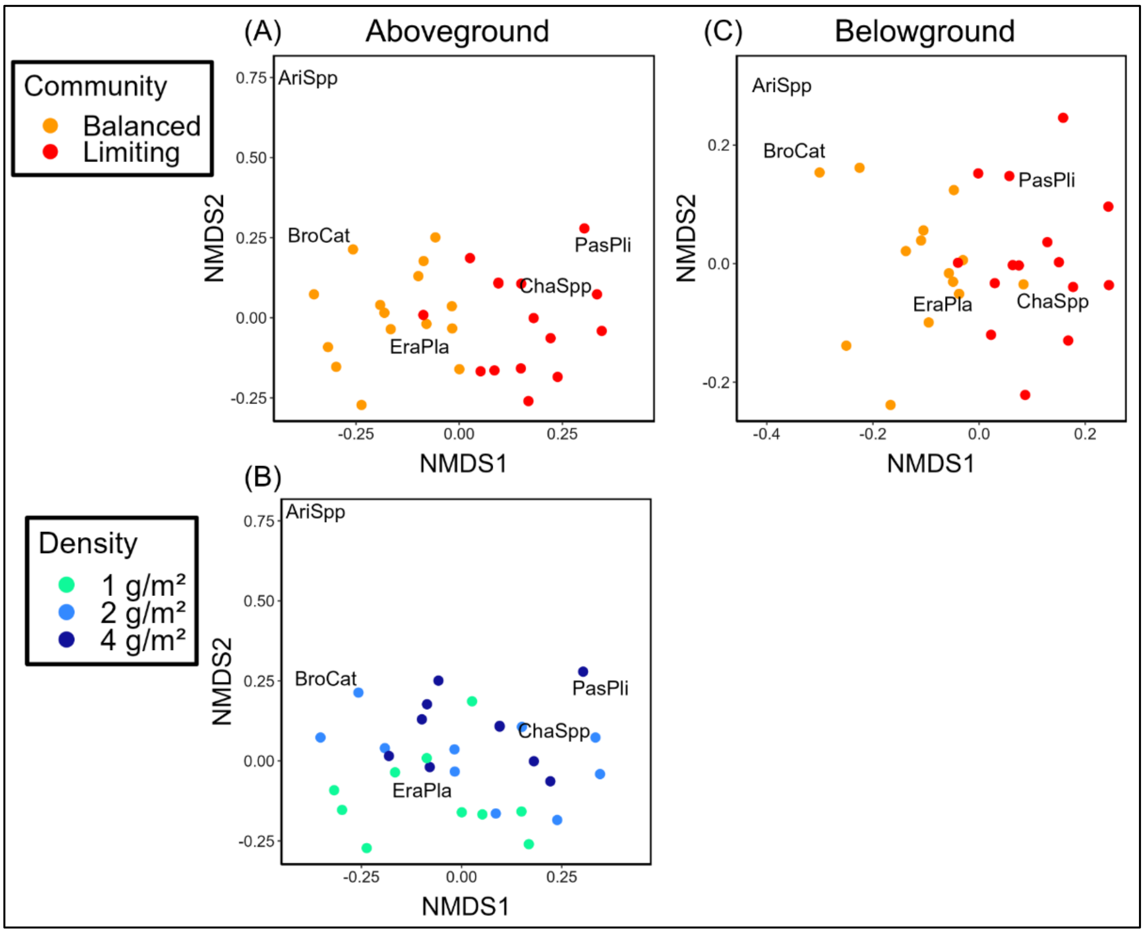

Figure 2. NMDS ordination plots to community composition with aboveground biomass according to Community factor (A) and Density factor (B) and belowground biomass according to Community factor (C). AriSpp = *Aristida* spp.; BroCat = *Bromus catharticus*; ChaSpp = *Chascolytrum* spp.; EraPla = *Eragrostis plana*; PasPli = *Paspalum plicatulum*.

Discussion

Eragrostis plana Biomass

Our nine-month greenhouse experiment, where two different seed mixes were sowed at three densities to outcompete the invasive grass *E. plana*, demonstrates the strength of this invasive species. This invasive species had higher biomass both aboveground and belowground than all native species together, regardless of the sowed community and the density of sowing. Invasive biomass (aboveground and belowground) was lower in communities with native species compared to *E. plana* monoculture. However, contrary to our first hypothesis, the Limiting community did not perform better than the Balanced community in reducing *E. plana* biomass: both sowed communities did not differ from each other. Similar studies also showed no or little support that communities designed following the limiting similarity theory increase invasion resistance (Hess et al., 2019). To create a community that overlaps all dimensions of the niche of an invader seems to be, at least, hard or even unrealistic (Hess et al., 2019). The community created by the method that we used here was restricted in maximizing trait similarity within the space of a limited set of traits (i.e., niche dimensions) of the species pool chosen, a limitation resulted from practical constraints and not by the method itself (Yannelli et al., 2018). Also, it is common that the established communities are different from the seed mixes designed (Kaul & Wilsey, 2023), as occurred in our study. Other functional traits could be more relevant in the early stages of plant establishment; here, no "juvenile traits" (related to competition in the early stage) were considered to design the limiting community, which might contribute to a failure in niche overlapping in the early stages (Yannelli et al., 2018). So, in our experiment, the established communities were quite different from the sowed seed mixes, either the Balanced community or the Limiting community that should have overlapped the *E. plana* niche. This fact limits our capacity to judge if communities based on limiting similarity are better or not competing with the invader in comparison to communities that did not consider such a feature. Our communities were different because some species germinated or established badly in the pots. The reasons for this are not known.

We also hypothesized that sowing higher seed densities of native species would negatively impact the biomass of the invader. Our data confirm this only for aboveground biomass, despite significantly higher below and aboveground biomass for native species at the highest seeding density. So, higher seed densities here were better to compete with invasive species, as also observed by other studies (Csákvári et al., 2023; Rojas-Botero et al., 2022; Yannelli et al., 2017, 2018). In Möhrle et al. (2021), all six designed seed mixes suppressed the invader at the same level in a greenhouse experiment, no matter how the seed mix was designed. This highlights that the first concern should be to introduce native species to occupy the space (Möhrle et al., 2021), irrespective of specific seed mixes designed purposes. In a meta-analysis, Clark et al. (2007) found that, in seed addition experiments only 15% of added seeds can be established as seedlings. Unfortunately, we did not control the exact number of added seeds and those that germinated to make a comparison. Thus, using high-density sowing should be a priority step in invader control projects. However, sowing more seeds represents a higher cost for restoration projects. Seed cost is the second most important factor determining seed mixes in grassland restoration projects, behind seed availability (Barak et al., 2022). In Campos Sulinos grasslands, the use of seeds, at the moment, depends almost exclusively on seeds collected manually, and higher seed densities mean more field effort to collect seeds.

No differences between the three sowing densities on invasive belowground biomass was found, although we showed an increase in native belowground biomass with increasing densities. In greenhouse experiments, often it is hard to negatively affect the invasive belowground biomass using different native species (e.g., Broadbent et al., 2018; Garrett & Gibson, 2020). Belowground competition can be more important driving invasive plant success than aboveground competition (Scherber et al., 2010; Broadbent et al., 2018).

Native Biomass and Native Species Performance

Regarding the biomass of native species, there was no difference between the two communities in terms of aboveground biomass while there was a slightly, but significantly higher belowground biomass production of the Limiting community. Higher above and belowground biomass production at the two higher-density sowing treatments reflect, once again, that it is more important to add a high number of seeds, no matter what the proportion of the species seeded.

Similarly to other studies (e.g., Daneshgar & Jose, 2009; Csákvári et al., 2023), our results seem to reflect the good performance of some particular species: *P. plicatulum* (at Limiting community) and *B. catharticus* (at Balanced community). Our results indicate that both species present a potential for use in future restoration projects. Importantly, both species are also interesting to land managers due to their high forage values for livestock (Nabinger & Dall'Agnol, 2019), which increase the potential for using these species. However, they differ regarding their metabolism and phenology: *B. catharticus* is an annual (or bi-annual) C3 species and can play an important role outcompeting with *E. plana* in late winter, spring, and even early summer. On the other hand, *P. plicatulum* is a C4 grass, for which the peak of productivity is in summer, and which can compete with *E. plana* during summer and early autumn.

Normally, communities that establish at restoration sites have higher SLA values than the designed seed mixes that were used in restoration (Kaul & Wilsey, 2023), which is related to a high ability to allocate resources in new leaves and occupy space in the establishing plants (Pérez-Harguindeguy et al., 2013). Consequently, in the first phases of a restoration project, we should pay more attention to species with fast leaf development (higher SLA values) (Hess et al., 2020; Kaul & Wilsey, 2023; Yannelli et al., 2018) than to specific combinations of species with different trait (leaf or not) expressions, i.e., on creation of communities with specific functional features (e.g., Möhrle et al., 2021). In our case, the two species that were best established, *B. catharticus* and *P. plicatulum*, had the highest SLA values, which is in concordance with previous studies. *A. jubata* and *A. laevis* have low SLA values and presented a low biomass development. These species frequently present high germination rates (e.g., Guido et al., 2017; Roitman, 2021), but the slow biomass production in our experiment (see also Guido et al., 2019 and Roitman, 2021) discourages their use, at least initial phases of restoration.

At the three density sowing levels, the values of native belowground biomass were very low. Field experiments with seed addition also obtained low belowground biomass values for native species, including to Campos Sulinos native species (Giles et al., 2022; Silva, 2019), and the low investment in belowground biomass may limit restoration success (Giles et al., 2022). Root functional traits such as specific root length and root length density can be good proxies for plant belowground competitive abilities (Pérez-Harguindeguy et al., 2013; Ravenek et al., 2016) and could also be used to define potential species to first steps on restoration. Although root traits are hard to measure (Funk et al., 2017), data on root traits for species of the Campos Sulinos grasslands could help chosen species that might establish or develop better under harsh conditions of most degraded areas.

Limitations in knowledge on seed introduction and ways forward

A limited understanding of dormancy and germination behavior can hamper restoration efforts (Kildisheva et al., 2020; Vitis et al., 2020). The main plant families in Campos Sulinos grasslands are Poaceae, Cyperaceae, Asteraceae, and Fabaceae (Boldrini et al., 2015). It is well known that many species of the first three families have seeds with physiological dormancy, while legume species present physical dormancy (Baskin &

Baskin, 2014; Kildisheva et al., 2020). Unfortunately, dormancy of native grassland species in the Campos Sulinos grasslands has achieved little attention in research (Vieira et al., 2015), even though dormancy might affect restoration results. In addition, seeds used here were collected in 2020, 2021 and 2022. We do not have information about changes in seed longevity with time of storage, which can present a great variation according to species (Baskin et al., 2006; Vitis et al., 2020). Increasing our knowledge about seed biology (stocking, germination, dormancy, etc.) and seedling biology (establishment and growth) is crucial for the development of restoration ecology in the Campos Sulinos region (Barak et al., 2018; Ladouceur et al., 2018; Saatkamp et al., 2019).

Here, we only used grasses where seed collection is easier. It is essential to improve our knowledge about the use of other life forms, especially forbs. In grassland restoration (including in seed mix designs) managers normally select too many grasses to the detriment of forbs (Kaul & Wilsey, 2023; Silveira et al., 2020), as we did. Forbs are important elements of grasslands, for taxonomic and functional diversity, as well as ecosystem functioning (Grman et al., 2021; Silveira et al., 2020). However, in tropical and subtropical grasslands, such as in the Campos Sulinos, many forbs are slow-growing and have a low ability for reproduction from seeds (Giles et al., 2022; Overbeck & Müller, 2018; Veldman et al., 2015). Future research on seed introduction should include representatives of this group. In addition, other ways of species introduction, such as topsoil transfer, and seedling and turf transplantations, also should be considered and tested. These techniques benefit slow-growing species or species with high clonal growth (and low reproduction by seeds) (Giles et al., 2022; Mudrák et al., 2018) that might not perform well when relying on germination.

In the restoration of areas invaded by *E. plana*, or other aggressive invaders, designing seed mixes based on limiting similarity is still a challenge. We have considered traits that might influence the performance of plants on established communities and those that are currently available in plant trait-data of native species. As a result, the established communities were very different from those that were designed, and many constraints contributed to this. So, our results confirm that higher seed density is better for competing with invader species and that the performance of specific native species (*B. catharticus* and *P. plicatulum*) has shown some potential to compete with such aggressive invader. This makes the development of alternative techniques, or even of combinations of different approaches, necessary, when the task is to restore communities under high pressure of grass invasion.

Acknowledgements

We thank the staff at Parque Nacional da Lagoa do Peixe and Parque Natural Municipal Saint'Hilaire, where we collected the larger part of used seeds, and the staff of Jardim Botânico de Porto Alegre, where we carried out the experiment. We also thank all colleagues who helped with fieldwork, experiment setup and maintenance, and data analysis, especially Alice Roitman.

Funding information

This work was supported by Funbio and Instituto Humanize (Bolsas Funbio - Conservando o Futuro, to PAT), by CNPq (grants 200657-2022/4 to PAT and 432356/2018-5 to GEO), and by Fundação Boticário (grant 1132_20182 to GEO). PAT and ABP were financed by CAPES (Finance Code 001). GEO is supported by CNPq (310345/2018-9 and 304852/2022-8).

Author contributions

PAT, GEO, and SCM conceived of the research idea; PAT collected the data; PAT performed the statistical analyses; PAT, EB, GEO, and SCM discussed the results and wrote the manuscript.

Data availability

Dataset and analysis codes are available at Zenodo Repository (Thomas et al., 2023).

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Supplementary Material

Table S1. Emergence data values and the origin of the seeds used in the experiment.

Species	Emergence (%)	Seed source
Anthenantia lanata	77 (Roitman, 2021)	Parque Natural Municipal Saint'Hilaire, Viamão, Brazil
Aristida jubata	76 (Roitman, 2021)	Parque Natural Municipal Saint'Hilaire, Viamão, Brazil
Aristida laevis	90 (Roitman, 2021)	Parque Natural Municipal Saint'Hilaire, Viamão, Brazil
Axonopus affinis	96 (Isla Sementes)	Commercial lot (Isla Sementes)
Bromus catharticus	92 (Supp. Mat 1)	Campus do Vale, UFRGS, Porto Alegre, Brazil
Chascolytrum subaristatum	98 (Supp. Mat 1)	Parque Nacional Lagoa do Peixe, Mostardas, Brazil
Chascolytrum uniolae	94 (Supp. Mat 1)	Parque Nacional Lagoa do Peixe, Mostardas, Brazil
Paspalum notatum	70 (Colonial Sementes)	Commercial lot (Colonial Sementes)
Paspalum plicatulum	67 (Roitman, 2021)	Parque Nacional Lagoa do Peixe, Mostardas, Brazil
Eragrostis plana	68 (Roitman, 2021)	Invaded grasslands and roadsides in Rio Grande do Sul, Brazil

Supplementary Material 1

Emergence tests

We performed emergence tests for collected seeds of *B. catharticus, C. uniolae,* and *C. subaristatum* in a greenhouse, in Jardim Botânico of Porto Alegre, Brazil. Seeds were deposed in seedling trays filled with sterilized soil and *Carolina Soil*® (a commercial mixture of turf and vermiculite) and then covered with a fine layer of substrate. For each species we had five replicates, with 10 seeds each replicate. Seedling trays were frequently moved and irrigated. Emergence tests were set up on April 25, 2022, and monitored for 60 days, and emergence values are presented in Table S1.

Table S2. Leaf trait values used to calculate the Limiting community, according to model suggested by Laughlin (2014)

Species	LA	SLA	LDMC	Source
Anthaenantia lanata	664.51	9.74	332.87	Silva et al. 2020
Aristida jubata	170.07	4.58	546.18	Levcamp Trait Data Bank (unpublished data)
Aristida laevis	715.93	6.55	487.68	Levcamp Trait Data Bank (unpublished data)
Axonopus affinis	535.80	23.02	258.72	Levcamp Trait Data Bank (unpublished data); Silva et al. 2020
Bromus catharticus	1513.84	20.71	224.43	Our measurement
Chascolytrum subaristatum	644.33	15.64	346.80	Hoss et al (under review); Levcamp Trait Data Bank (unpublished data)
Chascolytrum uniolae	1103.82	14.41	304.38	Our measurement
Paspalum notatum	560.62	22.36	286.32	Hoss et al (under review); Levcamp Trait Data Bank (unpublished data)
Paspalum plicatulum	661.15	15.24	282.26	Levcamp Trait Data Bank (unpublished data)
Eragrostis plana	682.67	13.27	330.86	Hoss et al (under review); Levcamp Trait Data Bank (unpublished data)

Table S3. Dry weight of 1000 seeds. Measured according to Pérez-Harguindeguy et al. (2013).

Species	Weight (g)
Anthaenantia lanata	1.129
Aristida jubata	7.194
Aristida laevis	1.916
Axonopus affinis	0.292
Bromus catharticus	9.316
Chascolytrum subaristatum	1.016
Chascolytrum uniolae	0.277
Paspalum notatum	1.356
Paspalum plicatulum	1.859
Eragrostis plana	0.198

Table S4. Number of added seeds (and percentage over the total number of native added seeds) in each treatment according to the weight of 1000 dry seeds (Table S3). Note that these are values for one sowing, and we sowed each native species twice (and the invasive species once).

	Limiting			Balanced		
	1 g/m^2	2 g/m^2	4 g/m^2	1 g/m^2	2 g/m^2	4 g/m^2
Anthaenantia lanata	35 (27)	70 (27)	139 (27)	11 (8)	23 (8)	46 (8)
Aristida jubata	1 (1)	2 (1)	4 (1)	2 (1)	4 (1)	8 (1)
Aristida laevis	3 (2)	6 (2)	12 (2)	7 (5)	13 (5)	27 (5)
Axonopus affinis	28 (22)	55 (22)	111 (22)	44 (31)	88 (31)	176 (31)
Bromus catharticus	1 (1)	2 (1)	4 (1)	1 (1)	3 (1)	6 (1)
Chascolytrum subaristatum	8 (6)	16 (6)	32 (6)	13 (9)	25 (9)	51 (9)
Chascolytrum uniolae	33 (26)	67 (26)	134 (26)	46 (33)	93 (33)	185 (33)
Paspalum notatum	4 (3)	9 (3)	17 (3)	9 (7)	19 (7)	38 (7)
Paspalum plicatulum	14 (12)	29 (12)	57 (12)	7 (5)	14 (5)	28 (5)
Total of native seeds	128	255	510	141	281	563
Eragrostis plana	292 (2.29)	292 (1.14)	292 (0.57)	292 (2.08)	292 (1.04)	292 (0.52)

Table S5. Mean dry biomass of *E. plana* and the total native biomass per level factor and significance according to Anova. † Control treatments were not included in the Anova. In bold, significant *p* values.

	Invasive biomass		Native biomass	
	Aboveground	Belowground	Aboveground	Belowground
Control	145.26†	64.12†	-	-
Community	*F* = 1.081, *p* = 0.308	*F* = 2.679, *p* = 0.114	*F* = 2.109, *p* = 0.159	*F* = 5.921**,** ***p* = 0.022**
Balanced	94.13	36.56	25.09	5.08^{b}
Limiting	102.84	42.74	30.52	7.01^{a}
Density	*F* = 4.155, ***p* = 0.028**	*F* = 0.109, *p* = 0.897	*F* = 5.170, ***p* = 0.009**	*F* = 5.966, ***p* = 0.007**
1g	114.38^{a}	40.90	19.28^{b}	4.42^{b}
2g	95.94^{ab}	39.01	29.74^{ab}	5.94^{ab}
4g	85.14^{b}	39.05	34.38^{a}	7.77^{a}
Interaction	*F* = 1.543, *p* = 0.234	*F* = 2.884, *p* = 0.075	*F* = 1.398, *p* = 0.266	*F* = 2.313, *p* = 0.120

Table S6. *Post-hoc* pairwise comparison (Tukey test) between density sowing levels on *Eragrostis plana* aboveground biomass. In bold, significant *p* values.

Contrast	Diff	Lower	Upper	*p* adj
1 g/m^2 - 2 g/m^2	-18.444	-44.066	7.178	0.191
1 g/m^2 - 4 g/m^2	-29.247	-54.869	-3.624	**0.023**
2 g/m^2 - 4 g/m^2	-10.803	-36.425	14.819	0.551

Table S7. *Post-hoc* pairwise comparison (Tukey test) between density sowing levels on native aboveground biomass. In bold, significant *p* values.

Contrast	Diff	Lower	Upper	*p* adj
1 g/m^2 - 2 g/m^2	10.462	-0.973	21.898	0.077
1 g/m^2 - 4 g/m^2	15.105	3.669	26.541	**0.008**
2 g/m^2 - 4 g/m^2	4.642	-6.793	16.078	0.575

Table S8. *Post-hoc* pairwise comparison (Tukey test) between density sowing levels on native belowground biomass. In bold, significant *p* values.

Contrast	Diff	Lower	Upper	*p* adj
1 g/m^2 - 2 g/m^2	1.514	-0.902	3.932	0.279
1 g/m^2 - 4 g/m^2	3.332	0.921	5.756	**0.005**
2 g/m^2 - 4 g/m^2	1.824	-0.593	4.242	0.164

Table S9. Final relative abundance (%) of the biomass of each species over the total native biomass in each harvest. Dominant species in each community is written in bold.

Biomass	Aboveground		Belowground	
Species	Balanced	Limiting	Balanced	Limiting
Aristida spp.	>1	0	>1	0
Bromus catharticus	**75**	17	**63**	15.5
Chascolytrum spp.	1	1.5	>1	1

Paspalum plicatulum	24	**81.5**	36	**83.5**

Table S10. *Post-hoc* comparison to community composition performed with pairwise-Adonis (Arbizu, 2019). In bold, significant *p* values.

Biomass	Factor	Comparison	*F* value	*p* value
Aboveground	Community	Balanced × Limiting	7.213	**< 0.001**
	Density	1 g/m² × 2 g/m²	0.161	0.166
		1 g/m² × 4 g/m²	0.040	**0.049**
		2 g/m² × 4 g/m²	0.326	0.327
Belowground	Community	Balanced × Limiting	4.966	**< 0.001**

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CAPÍTULO 4

Burn them all? Use and efficiency of fire as a tool for grassland restoration

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Gerhard Ernst Overbeck, Sandra Cristina Müller

Artigo a ser submetido na revista Restoration Ecology, na categoria Review Article

Title

Burn them all? Use and efficiency of fire as a tool for grassland restoration

Running head

Review on fire for grassland restoration

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PAT, ABP, GEO, and SCM conceived and designed the review; PAT reviewed the papers; PAT, EB, GEO, and SCM analyzed the data; PAT, EB, ABP, GEO, and SCM wrote and edited the manuscript

Abstract

Globally, grassland ecosystems have been seriously degraded by, per example, land use change, exotic species, and changes on disturbances regime. The ecological restoration of these ecosystems is fundamental to fight against climate change and biodiversity loss. Fire is an important component in evolution and management on several grasslands around the world. As well, fire can be a tool to restore grasslands. In this paper we present a systematic literature review to know how and where fire has been used as a tool in grassland ecological restoration and its efficiency. To do that, we collected the results (positive, negative, or null) of fire treatments considering five restoration objectives: (1) increase biotic diversity, (2) remove woody biomass, (3) control exotic species, (4) promote abiotic changes, and (5) improve ecosystem services. We discuss our results to the totality of data and considering major grassland types. Our review show that fire has been used mainly to restore Temperate grassland in North America. In general, there are more positive results than negative when using fire in grassland restoration, however, there are even more no-effect of fire. Fire is highly efficient to remove woody biomass, mainly in Temperate and Mediterranean grasslands. Fire also is efficient to control exotic species but is more efficient when combined with another technique. This efficiency is low in Tropical and Subtropical grasslands. Fire can be used as a tool in grassland restoration, but its efficiency is highly context dependent.

Keywords

Endogenous disturbances, Fire-prone ecosystems, Old-growth grasslands, Open ecosystem, Restoration ecology, UN Decade on Ecosystem Restoration

Introduction

Grasslands, including savannas, prairies, and steppes (henceforth referred to as "grasslands”), are ecosystems primarily characterized by the dominance of grasses and grass-like species and are among the most widely distributed terrestrial biomes, covering approximately a third of the terrestrial Earth’s surface (Bond & Parr 2010; Gibson 2009). These ecosystems are associated with great biodiversity, such as the 13,000 plant species found in the entire Brazilian Cerrado (Fernandes et al. 2016) or the world record of 89 plant species per square meter in the mountain grasslands in Argentina (Cantero et al. 1999). Moreover, they also provide several important ecosystem services, for example, carbon sequestration, food and forage production, water erosion control, and freshwater supply (Bengtsson et al., 2019; Zhao et al., 2020).

However, grasslands worldwide have been strongly degraded. Land use change has historically been the main historical reason (Gibson 2009; Petermann & Buzhdygan 2021), and grasslands continue to experience habitat conversion in recent decades (e.g., Sohl et al. 2012; Baeza et al. 2022; Carbutt & Kirkman 2022). At the same time, invasive species, soil fertilization, alteration of fire and grazing regimes, and climate change are also threatening grassland ecosystems (Gibson 2009; Buisson et al. 2019; Veldman et al. 2015). In face of the overall increase of degradation, ecological ecosystem restoration become important to ensure biodiversity conservation, ecosystem functioning, and ecosystem services (Clewell & Aronson 2006; IPBES 2018), as evidenced by the declaration of the decade 2021-2030 as the "UN Decade on Ecosystem Restoration". Grasslands restoration is a fundamental component to achieve this goal (Dudley et al. 2020; Török et al. 2021). In this context, summarizing the current knowledge on grassland restoration is an important step to promote and improve research and ecological restoration itself (Török et al. 2021).

Many grassland ecosystems have evolved with fire as a key element (Leys et al. 2018; Strömberg 2011). In fire-prone grassland systems, fire is a determinant factor in selecting species and shaping the vegetation, altering the proportion of woody species and individuals, changing the chemical and physical soil properties, affecting nutrient cycling and other ecosystem functions (Bond & Keeley 2005; McLauchlan et al. 2020; Parr et al. 2014). Fire can stimulate plant flowering (e.g., Fidelis & Blanco 2014; Fidelis & Zirondi 2021) and germination (Stradic et al. 2015; Lamont 2023). In addition, grasslands excluded from fire for a long time can experience a reduction in plant species richness (e.g., Uys et al. 2004; López-Mársico et al. 2020), arthropod richness and abundance (e.g., Goldas et al. 2022), and bird richness and abundance (Beal-Neves et al. 2020). Nevertheless, fire effects on abiotic and biotic conditions can vary greatly according to biome characteristics and fire regimes, i.e., fire frequency, intensity and magnitude (Gibson 2009; McLauchlan et al. 2020).

Fire was one of the first tools that humans used to manage and shape open ecosystems for their benefit (Bond & Keeley 2005), such as in the Mediterranean basin (Fernandes et al. 2013), in Central and South Brazil (Behling et al. 2007; Pivello 2011), Australia (Jones 2012), California (Keeley 2002) and Eastern North America (Ryan et al. 2012). Today, fire is used for management in many grassland ecosystems, with different objectives, such as improvement of forage quality for cattle (e.g., Vélez-Martin et al. 2015; Koyanagi et al. 2013), reduction of flammable fuel and thus the risk of harmful wildfires (e.g., Burrows & McCaw, 2013) or as part of biodiversity conservation efforts (e.g., Fuhlendorf et al. 2006). Also, fire is often cited as a tool used to restore temperate (Humphries et al. 2021) and tropical and subtropical grasslands (Buisson et al. 2019), for example, to control invasive plant species (Weidlich et al. 2020). However, the use of fire in ecosystem management is often discussed controversially, and fire may be understood – sometimes against available evidence – as a detrimental process even in fire-prone ecosystems where it is endogenous (Silveira et al. 2020; Tölgyesi et al. 2022). It is important to understand the long-term effects of the use of fire (Dudley et al. 2020; McLauchlan et al. 2020) in grasslands and how fire can be an effective tool in the ecological restoration of grasslands (Augustine et al. 2021; Dudley et al. 2020; Leys et al. 2018).

In this study, we conducted a review on the use of fire as a tool for the ecological restoration of grasslands around the world. First, we investigate where and how fire has been used for restoration purposes, then we discuss its efficiency regarding five different objectives: to (1) increase biotic diversity, (2) remove woody biomass, (3) control exotic species, (4) promote abiotic changes, and (5) improve ecosystem services. Finally, we discuss shortcomings and challenges to demystify the use of fire for grassland restoration, mainly in regions where the use of fire is not well established.

Materials and Methods

Bibliographic survey and first paper filtering

In August 2022, we carried out a search on Web of Science with the following syntax: TS = (((grassland* OR savanna* OR prairie* OR steppe* OR cerrado OR campo* OR meadow* OR rangeland* OR pastizal* OR prad*) AND (restor* OR recover* OR reveget*) AND (fire OR burn*))). A total of 2798 papers were returned from this search. After that, we screened these papers. To be included in our review, they needed to attend to three criteria: (1) Does the paper report results from an ecological restoration study case? (2) Does it make use of fire? And (3) was carried on in a grassland? We did not include studies developed in areas with a long history of restoration where authors did not clearly separate the effects of various restoration actions from that of fire (e.g., Adams et al. 2021; Catano et al. 2022). Also, we excluded studies where fire was applied as post-restoration management (e.g., Copeland et al.,2002; Catano et al. 2022; Bach & Kleiman 2021). At the end of this process, a total of 155 papers were selected to proceed with data collection.

First data collection

For these 155 papers (Fig. 1), we collected information on the publication year, geographic coordinates of each study site reported, country, biome, degradation cause, size of burned area, and burning season (Table 1). The data of publication year, burned area, and burning season were collected for each paper, therefore we have 155 observations. For the other parameters, we collected the information for the study sites. As papers can have more than one study site, the number of study sites (215) is higher than the number of papers reviewed.

When the exact geographic coordinates were not provided by the authors, we obtained the geographic coordinates of the nearest place provided. Major grassland types (Table 1) were adapted from the global biome classification by Dinerstein et al. (2017), by grouping grassland sites located in forest biomes together with climatically similar grassland biomes (see details in Table S1). When authors inform more than one burned area (for example, two different study sites), the average of those informed areas was calculated to obtain one value for each paper. For the burning season, we classified the burning dates informed in the papers as follows: Northern Hemisphere/Southern Hemisphere - January, February, and March as Winter/Summer; April, May, and June as Spring/Fall; July, August, and September as Summer/Winter; and October, November, and December as Fall/Spring. An example of a filled table for this first data collection is presented in Table S2.

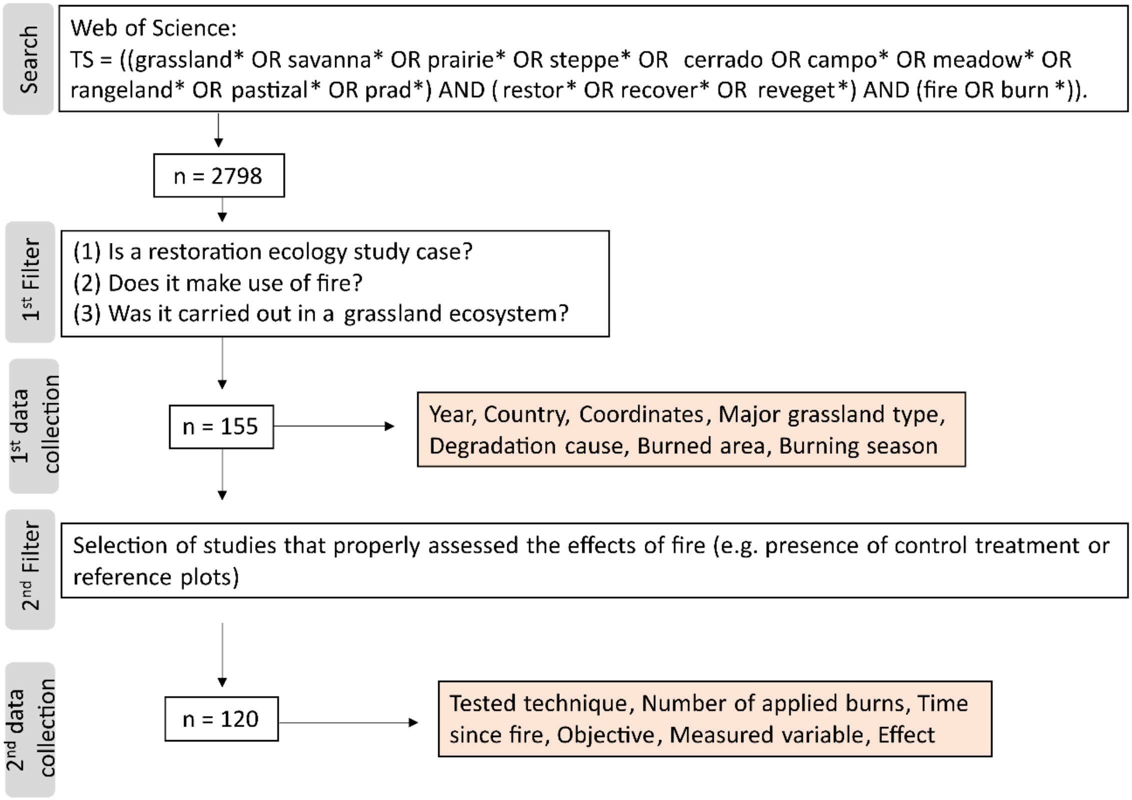

Figure 1. Flowchart of filter and data collection steps during the systematic review process.

Second paper filtering and second data collection

Then we ran a second filtering process, where we selected only the papers that properly assessed the fire effects and that clearly presented their results (Fig. 1). At this stage, papers, where the fire treatment was not compared with a control treatment, a reference treatment, and/or a pre-treatment condition, were disregarded. We also discarded papers that considered the fire treatment as the control treatment. At the end of this second filter, we selected 120 studies, that were submitted to the second data collection.

This new data collection was focused on the information about how the fire was applied, how it was evaluated, and its effects. We collected information about the tested technique (if the fire was applied alone or combined with another technique, and which technique), number of applied burns, time since fire between the applied burn and evaluation (in months), objective, response variable measured, and fire effect (positive, null, negative) (Table 1).

The same treatments applied in different seasons were considered as different treatments, for example, when fire was applied in Spring, or when fire was applied in the Fall. At this stage, each fire effect reported on each measured variable, time since fire, and study site was considered as a new observation. Therefore, the total number of observations depends on the study. When the data presented by the authors was not clear or highly variable or even not provided, we filled as “NA” (e.g., when authors did not inform the date of burning and the date of data collection, it was not possible to calculate the time between last burn and assessment). An example of a filled table for this data collection is presented in Table S3. The full list of response variables measured in the reviewed papers according to each objective is presented in Table S4.

Table 1. Overview of the collected data in our review. * more than one response can be signaled for the same observation.

Parameter	Response
Major grassland type	(1) Boreal grasslands; (2) Mediterranean grasslands; (3) Montane grasslands; (4) Temperate grasslands; (5) Tropical and Subtropical grasslands; (6) Xeric grasslands;
Degradation cause*	(1) land use conversion (e.g., agriculture; mining); (2) livestock intensification (e.g., sowing exotic forage); (3) alterations of regime disturbances (e.g., overgrazing; fire absence); (4) exotic species; (5) others

Burned area	(1) < 10 m²; (2) 16 m² - 64 m²; (3) 100 m² - 900 m²; (4) 0.1 ha - 1 ha; (5) 1 ha - 10 ha; (6) >10 ha
Burning season*	(1) Winter, (2) Spring, (3) Summer, (4) Fall
Number of applied burns	Numerical data (discrete)
Time since fire	Time (in months) between last applied burn and assessment
Tested technique*	Fire alone (0) or fire combined with: (1) species addition; (2) grazing; (3) herbicide; (4) herbaceous biomass removal; (5) physical barrier; (6) soil improvement; (7) woody biomass removal
Objective	(1) control exotic species, (2) improve ecosystem services, (3) increase species diversity, (4) promote abiotic changes, and (5) reduce woody encroachment
Variable measured	See Table 2
Effect	(1) positive; (2) null; (3) negative

Table 2. Description of objectives classified according to measured variables. The three objectives related to the biotic component (Increase species diversity, remove woody biomass, and control exotic species), can also include the measure of plant structures (flowers, bulks, stems), seed soil bank, and organism response to management (survival, recruitment, or mortality). See full list of measured variables for each objective in Table S4.

Objectives	Description
Control exotic species	Control exotic and invasive species (e.g., abundance, biomass, cover, species richness)
Improve ecosystem services	Increase different ecosystem services of interest (e.g., forage quality or quantity, erosion control)
Increase species diversity	Increase different diversity indexes (e.g., Shannon, Evenness) or their parameters (e.g., species richness, abundance, cover) to a single life form (e.g., forbs or bees) or all life forms (e.g., total plant cover).
Promote abiotic changes	Change environmental conditions to improve the environmental quality (e.g., physical and chemical soil conditions), or to facilitate plant establishment (e.g., remove woody debris or litter)

Reduce woody encroachment	Reduce the presence (e.g., abundance, biomass, cover) of woody component (trees, shrubs, stems)

Data Analysis

For the following data, we calculated the frequencies (number of papers out 155): year of publication, burned area, and burning season. For the data of major grassland type and cause of degradation we calculated the frequencies based on the number of study sites (number out 215). And for the data of tested techniques, number of applied burns, time since fire, objectives, and measured variable, we calculated the frequencies considering the total number of observations (number out 2126). The analysis for effects of fire considered if fire was tested alone or if was combined with another technique, the major grassland type, and objective. This analysis was limited to results reported up to 24 months after the last fire applied, as effects of other processes than fire can become more relevant after such a time period. Indeed, grassland structure, the cover of plant functional groups, and/or plant diversity return to pre-fire values or are not any more different to no-burned plots 24 months after a fire in different grassland ecosystems (Bahía & Zalba 2019; Vidaller et al. 2019; Wu et al. 2014).

All analyses and figures were performed in R (R Core Team 2023), except the map (Fig. 2), created using QGIS.org (2023), and the Sankey diagram (Fig. 6), generated at SankeyMATIC (https://sankeymatic.com).

Results

Where, when and why fire was applied?

The 155 papers reported data for 215 studied sites in six major grassland types (Table 3). A major part of the study sites was in Temperate Grasslands (75%), followed by Tropical and subtropical Grasslands (9%), Mediterranean Grasslands (7%), Xeric Grasslands (6%), Montane Grasslands (2%), and, lastly, Boreal Grasslands (lower than 1%, only one study site). Due to the low number of papers and observations, we do not present the results on Montane and Boreal grasslands when discussing the fire effects per major grassland type. Most of the papers reviewed was developed in the USA (79%; Fig. 2).

Across the 215 studied sites, the presence of exotic species (42%) was the most frequent cause of degradation, followed by alterations of the disturbance regime (31%), livestock intensification (13%), land use conversion (10%), and other (3%) (Table 2).

More than one type of degradation was reported to 26% of study sites. Regarding the burning season, 31 (20%) papers were reported burning in Winter; 45 (29%) during Spring, 23 (15%) during Summer, and 43 (28%) in the Fall. Eighteen papers reported burning in two different seasons (11%). In 17 (11%) papers the burning date was not informed or not clear (for example, “growing season”).

Table 3. Number of study sites (215) according to the degradation causes reported for each major grassland type.

Major grassland type	Exotic species	Disturbance regime changes	Livestock intensification	Land use conversion	Other	Total
Boreal	0	0	1	0	0	1
Mediterranean	13	3	1	1	2	20
Montane	0	3	3	0	0	6
Temperate	90	66	17	20	6	199
Tropical and Subtropical	9	5	6	6	0	26
Xeric	4	10	8	0	1	23
Total	116	87	36	27	9	275

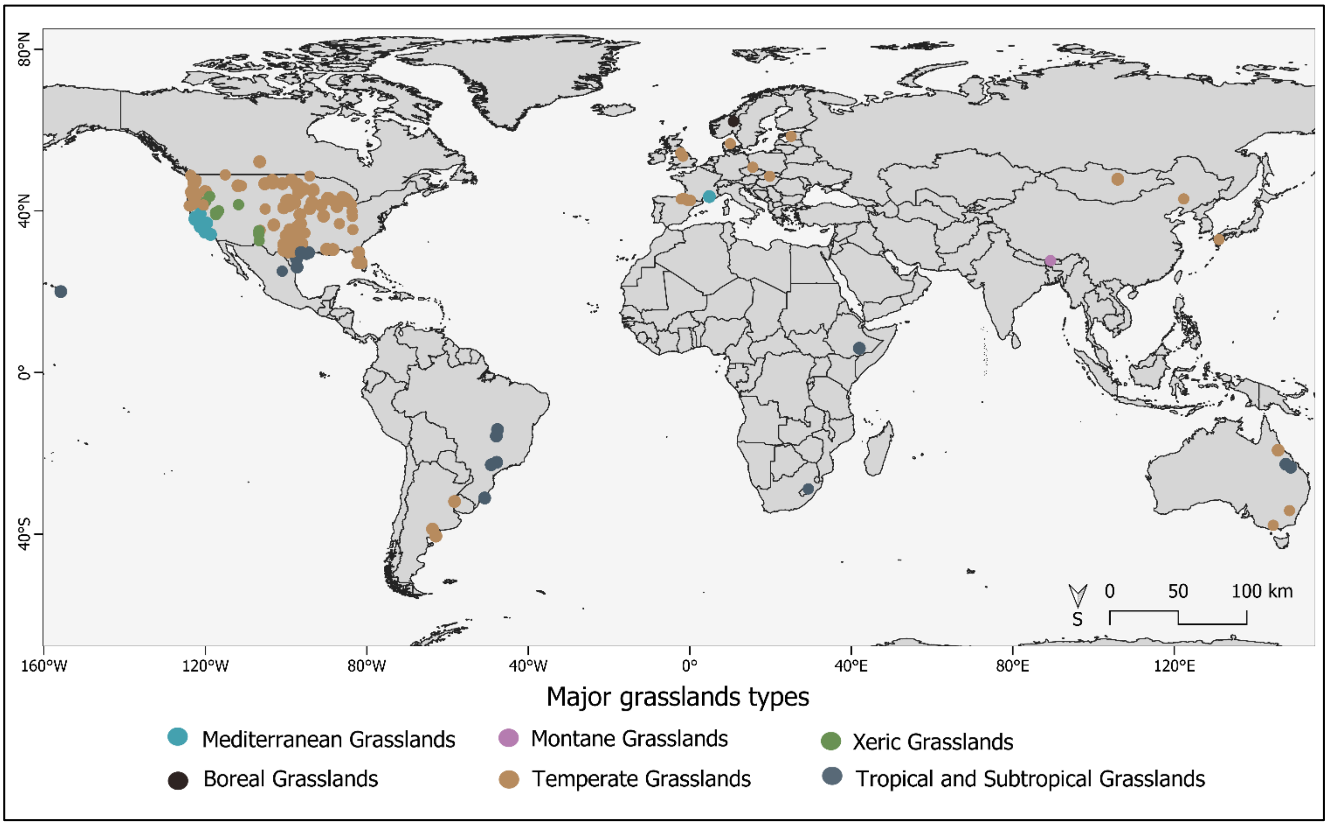

Figure 2. Location of the 215 study sites at the major grassland types, adapted from Dinerstein et al., (2017). See Table S1 for more details.

About half of the reviewed papers was published after 2011 to 2020 (52%; Fig. 3A). The burned areas at the 215 study sites varied from small experimental scales (1 m²) to the landscape scale burns (up to 334 ha in van Mantgem et al. (2021) (Fig. 3B), there were twice as many studies on small areas (< 1 ha) than on larger areas (> 1 ha).

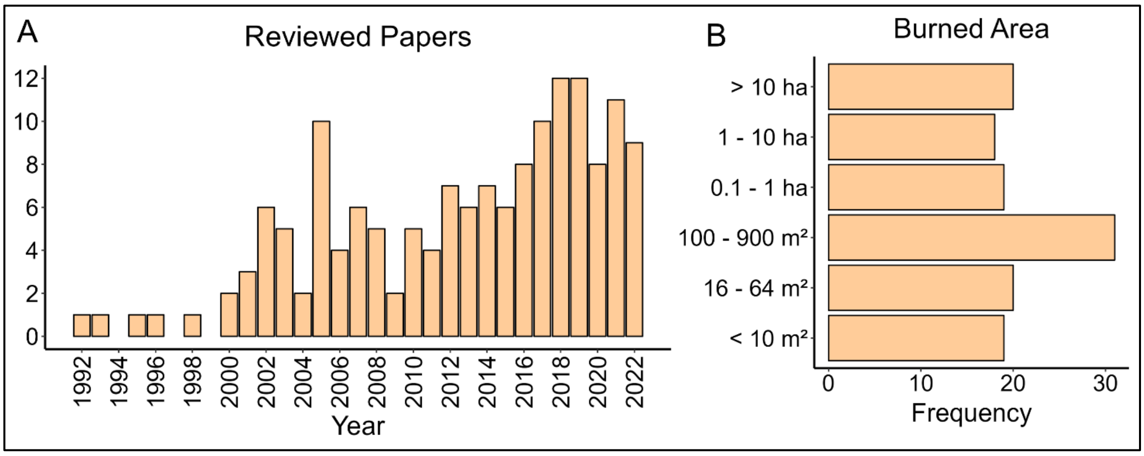

Figure 3. Number of papers depending on (A) the year of publication and (B) the size of the burned area reported in the 155 reviewed papers.

Fire Treatments: General data

In the second filter process, we obtained the results of the use of fire in grassland restoration from 120 papers, with a total of 2126 observations. Regarding the objectives, 50% of them were related to increasing species diversity, 21% to controlling exotic species, 10% to promoting abiotic changes, 10% to improving ecosystem services, and 9% to remove woody species (Fig. 4A). Seventy-nine percent (79%) of the studies reported results on one burn only (Fig. 4B), combined or not with another technique, and the maximum of applied burns was seven.

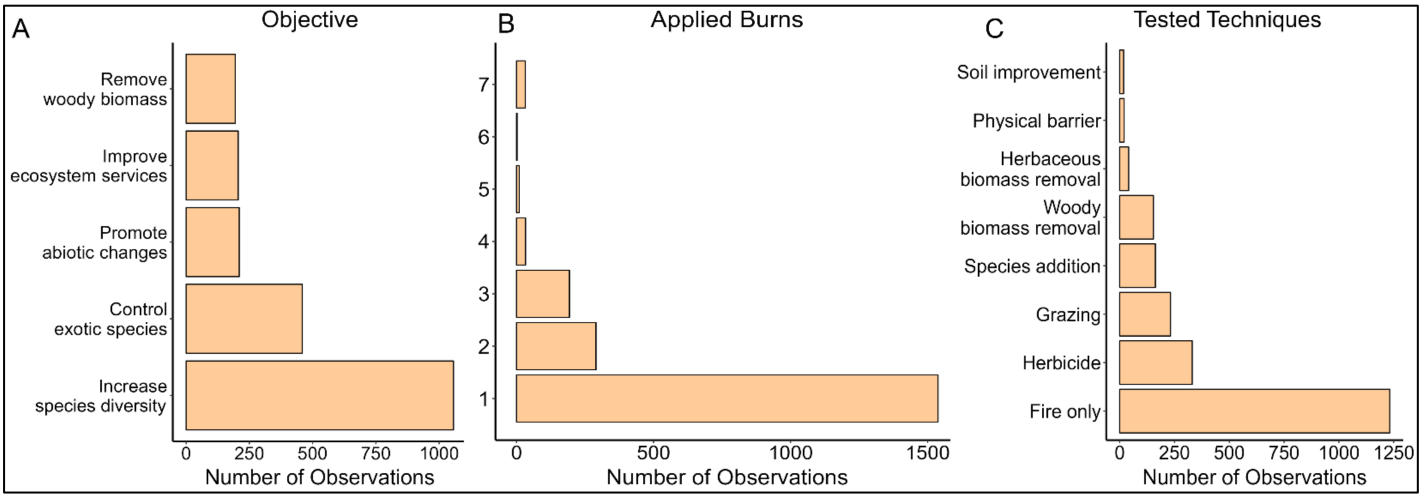

Figure 4. Number of observations depending on (A) objectives, (B) number of applied burns, and (C) fire only and fire with additional techniques tested out of the 2126 observations collected from the 120 papers.

Fifty-eight percent (58%) of the studies reported results of fire treatment alone (1224), then followed by techniques combined fire with: herbicide, grazing, species addition, woody biomass removal, herbaceous biomass removal, use of physical barriers, and soil improvement (33% of the total; Fig. 4C). Only 197 observations (9% of the total) are results for three (fire plus two) or more techniques. See the full table of tested techniques per grassland major type in Table S5.

Considering the total of 2126 observations, we obtained 1588 observations from Temperate grasslands, where 483 (30%) were positive and 164 (10%) negative observations but varying within the objectives and measured variables (and the remainder 1105 observations are null). For Tropical and Subtropical Grasslands, there was a total of 189 observations: 60 (32%) positive and 14 (7%) negative. In Mediterranean grasslands we collected 214 observations: 60 (28%) positive and 14 (6%) negative. And for Xeric grasslands, we found 104 observations: 10 (10%) positive and 10 negative (10%). The majority of observations was null.

Effects of fire for restoration of major grassland types

Fire alone as a technique

When fire was tested alone (time since fire up to 24 months) and regardless the grassland type and the objective, a large part of papers reported no effects, followed by positive and then negative effects (Fig. 5). The absolute number of positive and negative observations within this window of 24 months after fire in Temperate grassland studies was 195 (31%) and 82 (13%), respectively. For Mediterranean grasslands, we found 42 positives (37%) and nine negatives (8%), for Tropical and Subtropical grasslands 13 positives (23%) and seven negatives (13%), and for Xeric grasslands all observations reported no effects.

Considering the objectives (also considering time since fire up to 24 months but no the major grassland type), fire was highly effective to remove woody biomass, with 63 (70%) positive observations and only 2 (2%) negative observations. To exotic species control has 70 positive observations (35%) and 29 (14%) negatives. When the objective is to improve ecosystem services, 21 positives (27%) and 6 (8%) negatives. To increase species diversity, 84 observations was positive (24%) and negative in 31 (9%). To promote abiotic changes, only 12 results were positive (13%) while 30 (33%) negatives.

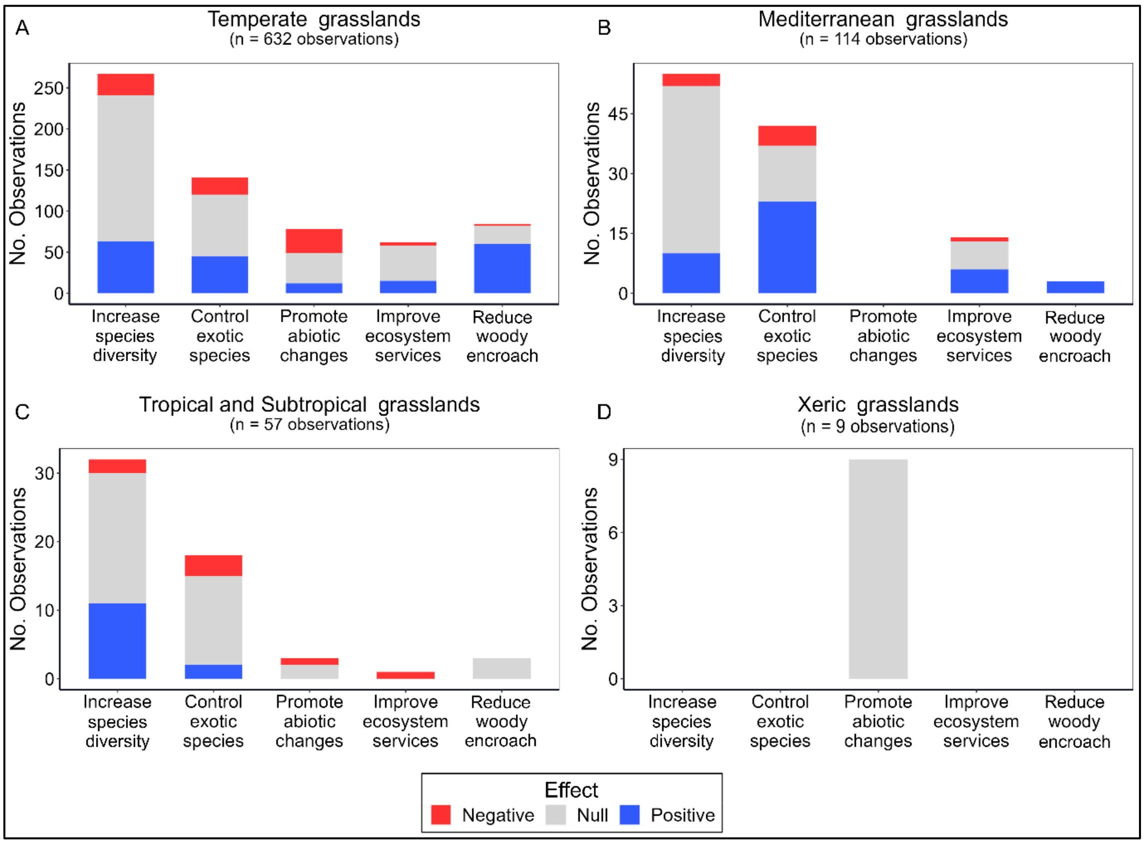

Figure 5. Number of observations of effects of fire alone used in grassland restoration for each objective in each major grassland type (considering time since fire up to 24 months).

Fire combined with other technique

When fire was combined with another technique (time since fire up to 24 months), we obtained 631 observations (30% of the total), and a large part of papers reported no effect, regardless the grassland type and the objective (Fig. 6). For Temperate grasslands we obtained 437 observations, with 140 positive (32%) and 28 negative (6%) observations. Tropical and Subtropical grasslands have 108 observations, where 40 were positive (37%) and 6 negative (4%). For Mediterranean grasslands, we collected 66 observations, with 12 positives (18%) and only one negative (1.5%). For Xeric grasslands, we obtained 20 observations with five positive (25%) and six negative (30%) observations.

The Sankey diagram (Fig. 6) shows the results (observations with positive, negative, or null effects on measured variables) considering the interrelation between the objectives and the respective techniques combined with fire for each major grassland type. For better visualization, we limited this diagram to those 462 observations of fire plus one technique (i.e., we disregarded those with fire plus two or more techniques – 197 observations). Also for better visualization, for Temperate Grasslands we present here only the situations with four or more observations (see diagram with all observations in Fig. S1 - see the full table of tested techniques in Table S5). There is a great variation in the objectives and techniques employed in Temperate grasslands with few negative effects, but the majority having either positive outcomes or no effects (Fig. 6A and Table S5). In Tropical and Subtropical grasslands, approximately half of observations are from studies that combined fire with species addition, a third of them with positive outcomes on biodiversity (Fig. 6B). The combination of fire with herbicide was also common with similar positive and negative effects being observed (Fig. 6B). In contrast, in Mediterranean grasslands and Xeric grasslands most studies combined fire with species addition with less than quarter of positive outcomes (Fig. 6C – D). In addition, for Xeric grasslands, the proportion of negative outcomes was greater than for other grassland types.

Regarding the objectives (considering time since fire up to 24 months and regardless the major grassland type and the technique), again fire was highly efficient to remove woody biomass, where 17 observations (71%) were positive and there were no negative results reported. To exotic species control, 77 (46%) observations were positives and 11 (7%) negative. When the objective was to increase species diversity, we collected 84 (24%) positives and 14 (4%) negative. To increase ecosystem services, 13 (26%) were positive and 10 (20%) negative. And to promote abiotic changes, 6 (17%) positive and also 6 (17%) negative.

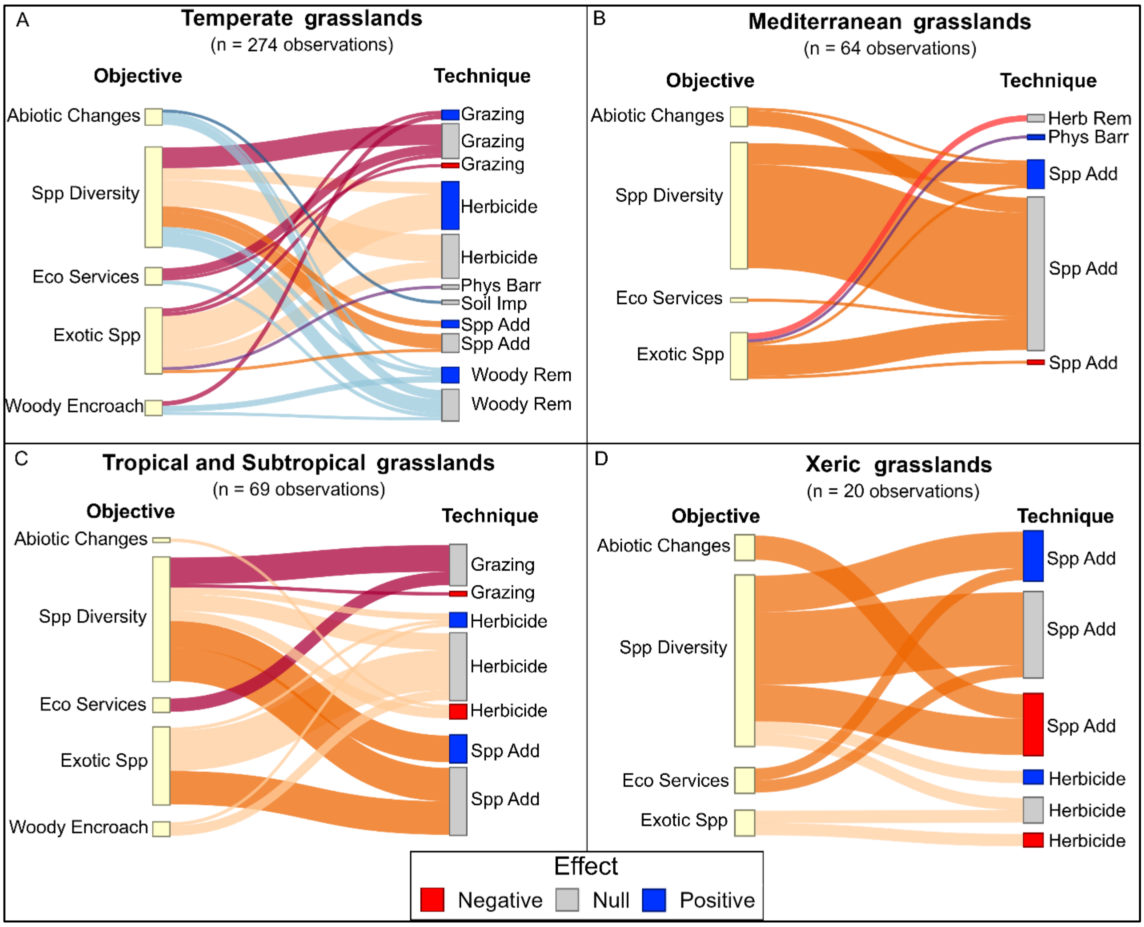

Figure 6. Effects of fire when combined with different techniques regarding different restoration objectives in Temperate grasslands (A), Mediterranean grasslands (B), Tropical and Subtropical grasslands (C), and Xeric grasslands (D), considering time since fire up to 24 months. Were plotted only those observations where fire plus one technique were used. Codes to objectives: Abiotic Changes = Promote abiotic changes; Diversity Index = Increase species diversity; Eco Services = Improve ecosystems services; Exotic Spp = Control exotic species; Woody Encroachment = Reduce woody encroachment. Codes to applied techniques (always in combination with fire): Grazing = Grazing; Herb Rem = Herbaceous biomass removal; Herbicide = Herbicide; Phys Barr = Physical barrier; Spp Add = Species addition (sowing or hay); Soil Imp = Soil improvement; Woody Rem = Woody biomass removal.

Discussion

Overall, more studies reported positive effects of fire than negative effects; however, even more studies showed no effect at all. Our review thus shows that fire can be a useful tool for the ecological restoration of grasslands, although its effects greatly vary with regard to the major grassland types, restoration objectives, and combination (or not) with other techniques.

We observed a growing tendency in the number of published papers over time, in concordance with other reviews about ecological restoration (Guerra et al. 2020; Yan & Liu 2021). This reflects the importance that ecological restoration has received in the last years. Most of the reviewed studies were carried out in North America, mainly in the USA, also in concordance with other reviews on ecological restoration studies (Kollmann et al. 2016; Weidlich et al. 2020; Ding et al. 2020). This is due to the long history of ecological restoration in this region of the world, but also due to the fact that the role of fire in many North American grasslands is well understood and that fire is often used as a management tool in conservation even on remnant grasslands (e.g., Collins & Wallace 1990; Fuhlendorf et al. 2009). The prevalence of studies in the Temperate grasslands in the North Hemisphere was related to degraded sites by exotic species. This region has been shown as more highly invaded by plants (Early et al. 2016; Seebens et al. 2015; Kleunen et al. 2015). On the other hand, there is a low number of studies on converted grasslands to other land uses, which probably means that these areas are not available for ecological restoration.

For Temperate grasslands, the grassland type with the highest number of studies, positive effects were more frequent than negative effects, except to promote changes in abiotic conditions (Fig. 5). Fire was especially efficient as a tool to reduce woody encroachment. When fire was used combined with another technique, positive effects were even more frequent than negative effects. In Mediterranean systems, all studies on the use of fire to reduce woody species showed positive effects. In a meta-analysis of woody removal in grasslands, Ding et al. (2020) found that the impact of removal can vary with the pretreatment encroachment levels, the removal method, or the traits of the target species. The ability to resprout for the woody species is a very important trait. Fire had no effect on the resprouter *Cornus drummondii* in the short term (six months; Lett & Knapp 2005), but other studies could control no-resprouter (Nuche et al. 2018; Alados et al. 2019) and resprouters (Ansley et al. 2006; Engber & Varner 2012; Hopkinson et al. 2020). When studies run for more years they show that resprouting shrubs can reach preburn values three or six years after fire (Fuhlendorf et al. 2011; Teague et al. 2010) but that fire can also maintain the species below the pre-fire values for a long time (8 years; Ansley & Castellano 2006). However, fire also stimulates some invasive legumes (e.g., Nuche et al. 2018; Sriramamurthy et al. 2020). Thus, the use of fire in restoration needs be planned with caution. Further, it appears to be important to also consider the need of continuing management with fire (or other techniques) in ecosystems with risk of woody encroachment even after the end of restoration activities (Buisson et al. 2021).

A high rate of positive effects of fire were found for exotic species control, especially in Mediterranean grasslands. These studies were focused mainly in controlling single exotic species from different life forms, such as the shrub *Genista monspessulana* (Alexander & D'Antonio 2003), the C3 grasses *Bromus diandrus* (Moyes et al. 2005) and *Taeniatherum caput-medusae* (Kyser et al. 2008), and the forb *Centaurea solstitialis* (Kyser & DiTomaso 2002). However, fire did not always have a positive effect on controlling *T. caput-medusae* in Mediterranean grasslands (e.g., Berleman et al. 2016; Kyser et al. 2008). In a review on exotic species management in Temperate grasslands, Humphries et al. (2021) found that studies that used only one treatment (not necessarily fire) were never highly successful in reducing weed biomass; but those using two or three treatments provided moderate effectiveness (60%), and those using four treatments, or more were always highly successful. Our results showed that combining fire with another technique seems to be a better strategy for exotic species control than using fire alone. For instance, we found that fire combined with herbicide have few negative effects on exotic species control in Temperate grasslands. Humphries et al. (2021) also found that combining herbicide and fire provided a very efficient control (90%) of exotic plants, compared to fire and herbicide when used alone (40% and 60% effectiveness, respectively). In Tropical and Subtropical grasslands, we found a general low efficiency of combining fire and herbicide to control exotic species, but it was even better than when using only fire. The general low efficiency of fire alone to control exotic species in Tropical and Subtropical grasslands can be consequence of fire adaptation of these exotic species: *Urochloa decumbens* (Assis et al. 2021), *Cenchrus ciliaris* (Marshall et al. 2012), and *Melinis minutiflora* (Martins et al. 2017) have a fast recovery and high biomass production after fire.

We observed a low number of positive effects reported on Xeric grasslands, both using only fire and when using fire combined with another technique. In dry grasslands, restoration success generally is better in wetter years (Buisson et al. 2021). For other ecosystems (grasslands and forests regardless of the climate), low precipitation has been reported as limitation to recovery after a fire or another restoration management (Giorgis et al. 2021; Barber et al. 2019; Groves & Brudvig 2019). This explains why fire is usually not an indicated technique for restoration of Xeric grasslands, as climatic conditions are extreme and consequently productivity is low (e.g., Ladwig et al. 2014). However, the low number of observations impedes a deeper discussion.

When considering fire alone to increase plant species diversity, its positive effects were geographically spread (in Temperate, Tropical and Subtropical, and Mediterranean grasslands) and on a wide range of variables: flowers (Lettow et al. 2014; MacDonald et al. 2013; Pavlik et al. 1993), C4 grasses (Ansley et al. 2022; Daehler & Goergen 2005), C3 grasses (Murray et al. 2021), forbs (Brambila et al. 2023; Reemts et al. 2021), or general plant species richness (Porto et al. 2022; Vidaller et al. 2019). Part of the non-positive effects on plant diversity increase can be a consequence of the fire application outside the historical range of the fire regime (Tangney et al. 2022). Human induced fires normally are applied outside the natural fire season (Bond & Keeley 2005; Platt et al. 2015), per example human fires during dry winter season in Brazilian Cerrado, contrasting with the lightning fires during the wet season (Ramos-Neto & Pivello 2000). This suggests that mimicking the natural fire regime may be a key factor when trying to restore grassland diversity, for example, burning in summer to Mediterranean grasslands (Buisson et al. 2021). Nevertheless, burning during the hotter seasons increases the probability of harmful wildfires and this should be considered when planning a prescribed fire. When considering effects on animals, fire (alone or combined) has positive effect on bees (Lettow et al. 2018), but no-positive effect on butterflies (Delaney et al. 2016), reptiles and frogs (Bower et al. 2014), birds (Fitzgerald & Tanner 1999), and small mammals (Jacques et al. 2017).

Directions to future studies

We excluded 35 studies that, while using prescribed fire in a restoration context, did not properly assess fire effects, did not include a control treatment (without fire) or a reference site to compare the results. It is important to carefully design experiments, including control and/or reference sites, to improve grassland restoration research and advance with restoration techniques (Török et al. 2021; Humphries et al. 2021). The definition of standardized experimental designs and assessment protocols would be interesting for a better understanding of fire effects in various ecosystems.

Furthermore, reviewed papers frequently did not collect data about fire severity and fire behavior, or even fire regime of the system. The inclusion of these data in the assessment is important for a better understanding of fire effects. In addition, a large part of studies assessed the impact of fire on plants (structure, individuals, or community). Expanding the assessment to other organisms and abiotic conditions should be considered to improve our comprehension of fire effects on ecological restoration. The claim for the inclusion of animal monitoring in ecological restoration and fire effects evaluation is not new (e.g., Kollmann et al. 2016; Pastro et al. 2014).

Define and apply an adequate fire regime to help us to manage and restore grasslands is a hard task (Pivello et al. 2021), which requires the work from different researchers and local communities (Driscoll et al. 2010; He et al. 2019; Bowman et al. 2020). It is important to keep in mind that climate change will increase fire frequency and intensity around the world (Bowman et al. 2020) and adequate fire regime management is necessary to reduce catastrophic fires (Pivello et al. 2021). Large gaps still remain regarding the best ways to use fire in grassland restoration, and further research need to address more specific questions.

Acknowledgments

PAT was financed by CAPES (Finance Code 001) and received a grant from CNPq (Finance Code 200657/2022-4) to develop part of this study at Avignon Université, France. ABP was supported by CNPq/MCTI/CONFAP-FAPS/PELD of 2020 and FAPESC 2021TR386. The authors declared that there are no conflicts of interest.

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Supplementary Material

Table S1. Major grassland types, adapted from biomes from Dinerstein et al. (2017), used to classify the reviewed papers.

Major grassland types	Biome according to Dinerstein et al. (2017)
Boreal Grasslands	Boreal Forests or Taiga
Temperate Grasslands	Temperate Grasslands, Savannas, and Shrublands Temperate Broadleaf and Mixed Forests Temperate Conifer Forests
Tropical and Subtropical Grasslands	Tropical and Subtropical Grasslands, Savannas, and Shrublands Tropical and Subtropical Coniferous Forests Tropical and Subtropical Dry Broadleaf Forests Tropical and Subtropical Moist Broadleaf Forests
Mediterranean Grasslands	Mediterranean Forests, Woodlands, and Scrub
Montane Grasslands	Montane Grasslands and Shrublands
Xeric Grasslands	Deserts and Xeric Shrublands
-	Flooded Grasslands and Savannas Mangroves Tundra

Table S2. Filled table as example of the collected data on the first data collection process.

authors	**paper**	**year**	**lat**	**long**	**major grassland type** (addapted from Dinerstein et al. 2017)	**Degradation Cause**					**class of burned area**	**burning season**	**properly assessed and reported the results**
						land use conversion	**livestock intensification**	**alterations on regime disturbances**	**exotic species**	**other**			
Abdalla, K; Muter	Grassland	2022	-28.811244	29.361075	Tropical and Subtropica	0	1	0	0	0	100 m² - 900 m²	fall	yes
Abella, SR; Mena	Rapid and	2020	41.553333	-83.835556	Temperate	0	0	1	0	0	1 ha - 10 ha	NA	no
Adams, CR; Galat	Increasing	2006	5.0291666	93.0497222	Temperate	0	0	0	1	0	100 m² - 900 m²	NA	yes
Adams, CR; Galat	Increasing	2006	44.862500	-93.600000	Temperate	0	0	0	1	0	100 m² - 900 m²	NA	yes
Alados, CL; Saiz,	CLEARIN	2019	2.6427777	0.12666666	Temperate	0	0	1	0	0	>10 ha	fall	yes
Alados, CL; Saiz,	CLEARIN	2019	42.602500	-0.181389	Temperate	0	0	1	0	0	>10 ha	fall	yes

Table S3. Filled table as example of the collected data on the second data collection process.

authors	**paper**	**major grassland type** (addapted from Dinerstein et al. 2017)	**tested technique**	**applied burns**	**time since fire** (in months)	**objective**	**effect**	**variable measured**
Abdalla, K; Mutema, M;	Grassland rehabilitation	Tropical and Subt	fire	3	36	Promote abiotic changes	NULL	soil (net CO2 emissions)
Abdalla, K; Mutema, M;	Grassland rehabilitation	Tropical and Subt	fire	3	36	Promote abiotic changes	NULL	soil (gross CO2 emissions)
Abdalla, K; Mutema, M;	Grassland rehabilitation	Tropical and Subt	fire	3	36	Promote abiotic changes	NEG	soil (organic carbon)
Abdalla, K; Mutema, M;	Grassland rehabilitation	Tropical and Subt	fire	3	36	Improve ecosystem services	NULL	plant (biomass)
Abdalla, K; Mutema, M;	Grassland rehabilitation	Tropical and Subt	fire	3	36	Promote abiotic changes	NULL	soil (density)
Adams, CR; Galatowitsc	Increasing the effectiven	Temperate	fire	1	1	Control exotic species	NEG	Phalaris arundinacea (shoot density)
Adams, CR; Galatowitsc	Increasing the effectiven	Temperate	fire	1	3	Control exotic species	NULL	Phalaris arundinacea (shoot density)
Adams, CR; Galatowitsc	Increasing the effectiven	Temperate	fire	1	12	Control exotic species	POS	Phalaris arundinacea (soil seed bank)

Table S4. Response variable measured and respective objectives.

Objective	Variable Measured
Control exotic species	*Acroptilon repens* (stems), *Amelichloa clandestina* (cover), *Avena spp.* (germination, cover), *Bothiochloa ischaemum* (cover, basal tillers/m², biomass, cover, frequency of crowns/m², frequency, reproductive tilles/m², tillers), *Brachypodium distachyon* (cover), *Bromus diandrus* (seedlings, soil seed bank), *Bromus inermes* (biomass, cover), *Bromus molliformis* (cover), *Bromus tectorum* (biomass), *Calamagrostis epigejos* (biomass, shoot height), *Cenchrus ciliaris* (cover), *Centaurea maculosa* (adults, biomass, juvenils, population, seedlings), *Centaurea solstitialis* (cover), *Cirsium arvense* (stems/m²), *Cyperus entrerianus* (cover, frequency), *Eragrostis lehmanniana* (density, soil seed bank), *Erodium botrys* (cover), *exotic forb* (cover, flowering plants, seeds, species richness), *exotic grass* (abundance, cover, seeds, species richness), *exotic legumes* (cover), *exotic plant* (biomass, cover, Shannon Index, species richness, soil seed bank), *Festuca arundinacea* (cover), *Festuca perennis* (cover), *Genista monspessulana* (soil seed bank), graminoid (flowering species), *Hypochaeris glabra* (cover), *Linaria dalmatica* (biomass, cover, density, seed production), *Melinis minutiflora* (biomass), *Paspalum notatum* (cover), *Phalaris arundinacea* (biomass, cover, height, shoot density, soil seed bank), *Pinus elliotii* (individuals), *Poa pratensis* (biomass, cover), *Potentilla recta* (large plants, small plants), *Taeniatherum caput-medusae* (biomass, cover, density, fecundity, seed rain), *Urochloa decumbens* (cover)
Improve ecosystem services	*Aristida purpurea* (biomass, cover), biomass quality, carrying capacity, exotic plant (cover), forb (biomass), grass (biomass, cover, density), herbaceous (biomass), legume (cover), plant (biomass, cover), sedge (cover), soil (organic carbon), soil erosion, unpalatable cactu (cover), unpalatable forb (cover), unpalatable plant (biomass, flowers, seedlings), unpalatable shrub (cover), water runoff
Increase species diversity	bee (abundance), bird (abundance, species richness), *Bromus* spp. (cover), bryophite (species richness), bulk (density), butterfly (community), exotic grass (cover), exotic plant (cover), flowering plants (abundance), forb (biomass, cover, density, flowering species richness, seeds, Shannon Index, soil seed bank, species richness), frogs and reptiles (abundance, species richness), gopher disturbance, graminoid (cover, flowering species, soil seed bank, species richness), grass (biomass, cover, density, inflorescence, species richness), herb (species richness), herbaceous (biomass, cover), *Heteropogon contortus* (cover, seedlings), legume (biomass, cover), mammal (community), meadow species (cover, species richness), *Nassella leucotricha* (cover), plant (abundance, biomass, community, cover, germination, indicator species, Shannon Index, Simpson diversity, soil seed bank abundance, species evenness, species richness, survival and growth, survival), sedge (biomass, cover, species richness), shrub (cover), soil seed bank (abundance, density, Shannon Index, species richness), woody species (cover)
Promote abiotic changes	bare soil (cover), dead plant (biomass), down woody debris, environmental structure, light availability, litter (cover), open sky, plant (height), soil (ammonium, C:N, carbon, cation exchange capacity, conditions and nutrients, conditions, density, electrical conductivity, exchangeable cations, gross CO_2 emissions, inorganic carbon, inorganic nitrogen, ion exchance, mineralizable nitrogen, moisture, net CO_2 emissions, NH_4, nitrate, nitrogen, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, NO_3, nutrients, organic carbon, organic matter, pH, phosporous, properties, temperature, water-extractable cations), thatch (cover)
Reduce woody encroachment	*Baccharis pilularis* (biomass), canopy openess, *Cornus drummondii* (cover), *Cornus sericia* (cover), crown area, *Echinospartum horridum* (biomass, seedlings), *Juniperus pinchotti* (cover), percent crown volume scorched, *Proposis glandulosa* (canopy cover), shrub (biomass, canopy cover, cover, density, height, mortality, recrutiment, stem density, mortality), tree (basal area, cover, density, diameter at breast height, mortality, seedlings abundance, species richness), woody species (canopy cover, cover, density, frequency, percentage, seedling survival, seedlings germinated, stems density, survival)

Table S5. Number of observations with each tested technique at the different major grassland types considering full time since fire data for evaluation.

Major grassland type	Fire alone	Grazing	Herbaceous Biomass removal	Herbicide	Physical barrier	Soil Improvement	Species addition	Woody biomass removal	Total
Boreal	20	0	0	0	0	0	0	0	20
Mediterranean	139	0	11	0	3	0	63	0	216
Montane	11	0	0	0	0	0	0	0	11
Temperate	918	205	8	266	17	14	190	154	1772
Tropical and Subtropical	73	13	22	65	0	4	59	0	236
Xeric	63	14	0	8	0	0	19	0	104
Total	1232	232	41	331	20	18	331	154	2359

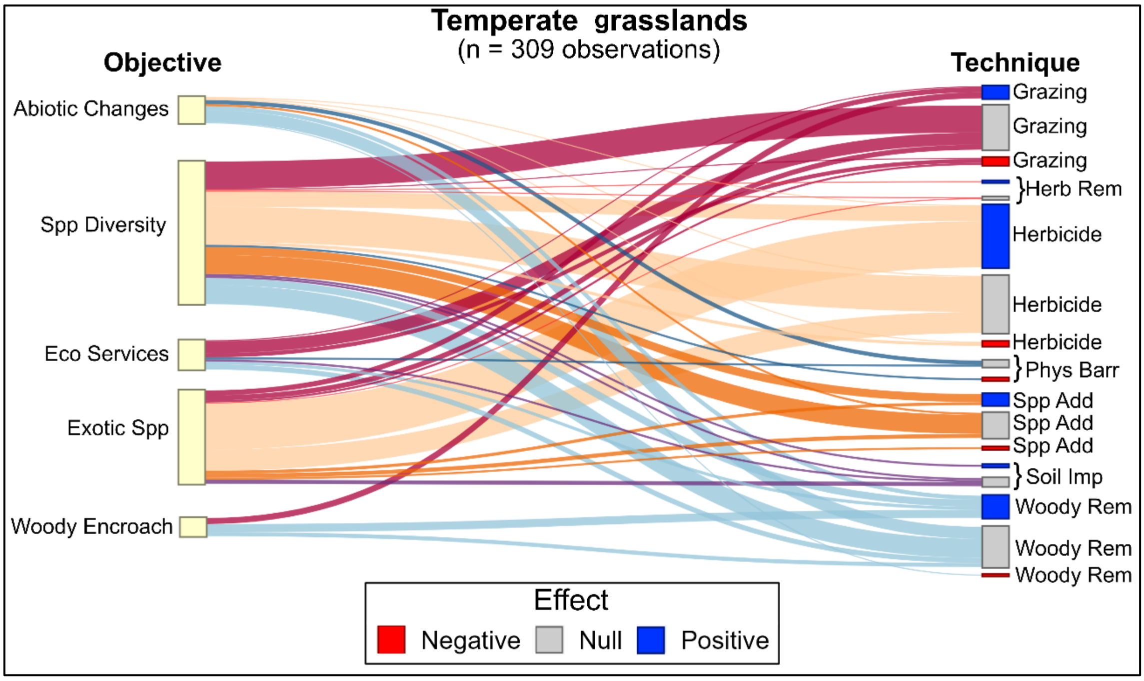

Figure S1. Sankey diagram to Temperate grasslands considering all observations to fire combined with an additional technique and time since fire up to 24 months. For better visualization, were plotted only those observations where fire plus one technique were used. Codes to objectives: Abiotic Changes = Promote abiotic changes; Spp Diversity = Increase species diversity; Eco Services = Improve ecosystems services; Exotic Spp = Control exotic species; Woody Encroachment = Reduce woody encroachment. Codes to applied techniques: Grazing = Grazing; Herb Rem = Herbaceous biomass removal; Herbicide = Herbicide; Phys Barr = Physical barrier; Spp Add = Species addition (sowing or hay); Soil Imp = Soil improvement; Woody Rem = Woody biomass removal.

CONSIDERAÇÕES FINAIS

Quando em 2019 iniciei o doutorado em Ecologia eu tinha muitas expectativas: saídas de campos, congressos, disciplinas em outros PPGs, quem sabe um doutorado-sanduíche. A vida acadêmica é movimento constante, algo que sempre gostei e foi parte importante na hora de escolher fazer um doutorado. Mas também sabia que seriam anos de muito trabalho acompanhados de estresse e pressão. O potencial que a vida acadêmica tem de bagunçar o psicológico daqueles que nela se arriscam já é bem conhecido. Para piorar, meu doutorado começava quase que ao mesmo tempo que um governo anti-ciência e anti-conservação, o que me fazia questionar se eu estava tomando a decisão certa de investir na carreira acadêmica naquele momento. Como desgraça pouca é bobagem, ainda houve uma pandemia. (Os problemas que o anti-governo e a pandemia trouxeram não precisam ser citados). Por diversas vezes foi muito difícil fazer este doutorado. Mas agora estou nas considerações finais da minha tese, o que praticamente encerra o doutorado. E o doutorado encerra um ciclo que se iniciou em 2010, quando entrei na UFSM para cursar biologia e que, desde o primeiro semestre, busquei trabalhar com restauração ecológica e controle de espécies invasoras. Por isso, não posso deixar de encerrar esta tese com este relato pessoal além de uma discussão dos artigos produzidos.

O doutorado é muito maior do que a tese apresentada neste arquivo. E, apesar das limitações, pude nestes 4 anos e meio participar de várias atividades que lá atrás imaginava realizar. Me envolvi em saídas de campos e trabalhos de diversos colegas e amigos em unidades de conservação do Rio Grande do Sul, no Pantanal e na França. E todas essas atividades foram importantes na minha formação como doutor em Ecologia. O doutorado-sanduíche na França, que felizmente pude realizar, foi desafiador e contribuiu muito no resultado desta tese. Estes 4 anos e meio de doutorado resultaram em 4 capítulos de tese, dois que se mantiveram desde o projeto apresentado na seleção do doutorado. Pois vamos às considerações finais:

A restauração ecológica dos Campos Sulinos ainda tem muito onde avançar, tanto em questões práticas como legais. Precisamos de avanços legais para impulsionar a prática além do realizado nas universidades. Ao mesmo tempo, pesquisas experimentais e toda pesquisa sobre conservação e manejo dos Campos Sulinos pode (e deve) ajudar a embasar questões legais para a restauração ecológica na região. As três técnicas discutidas mais a fundo nesta tese (transposição de feno, semeadura de espécies nativas e queima prescrita) podem ser úteis em diversos casos de restauração nos Campos Sulinos.

Entretanto, não há receita de bolo em restauração ecológica e cada caso deve avaliado. E por isso, o desenvolvimento da restauração ecológica deve ser contínuo e mais pesquisas são necessárias, principalmente com maior escala espacial-temporal. A introdução de espécies nativas em áreas degradadas e o controle de espécies invasoras seguem sendo atividades desafiadoras. E o tema da restauração ecológica é muito maior do que foi discutido nesta tese. Há pontos que apenas discutimos brevemente (como no primeiro capítulo) e podem render várias outras teses e dissertações. Custos e oportunidades econômicas da restauração ecológica devem ser abordados. A importância das pessoas (pecuaristas, comunidades tradicionais, indígenas e quilombolas) na restauração ecológica dos Campos Sulinos também deve ser abordada, principalmente devido ao potencial dos nossos campos nativos para o turismo e a produção sustentável.

Enfim, há muito trabalho pela frente.

Seguiremos pela restauração dos Campos Sulinos.

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Porto Alegre
2 de novembro de 2023