

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

**ESTRATÉGIAS E MECANISMOS ENVOLVIDOS NA DINÂMICA DE  
MANUTENÇÃO E REGENERAÇÃO DA VEGETAÇÃO CAMPESTRE**

**STRATEGIES AND MECHANISMS INVOLVED IN THE MAINTENANCE AND  
REGENERATION DYNAMICS OF GRASSLAND VEGETATION**

**Graziela Har Minervini Silva**

Orientador: Prof. Dr. Gerhard Ernst Overbeck

Porto Alegre

2020

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

**ESTRATÉGIAS E MECANISMOS ENVOLVIDOS NA DINÂMICA DE  
MANUTENÇÃO E REGENERAÇÃO DA VEGETAÇÃO CAMPESTRE**

**STRATEGIES AND MECHANISMS INVOLVED IN THE MAINTENANCE AND  
REGENERATION DYNAMICS OF GRASSLAND VEGETATION**

**Graziela Har Minervini Silva**

Tese apresentada ao Programa de Pós-Graduação em Botânica como um dos requisitos para a obtenção do grau de Doutor em Botânica pela Universidade Federal do Rio Grande do Sul.

Orientador: Prof. Dr. Gerhard Ernst Overbeck

Porto Alegre

2020

## AGRADECIMENTOS

“...Gracias a la vida, que me ha dado tanto  
Me ha dado el sonido del abecedario  
Con él las palabras que pienso y declaro  
Madre amigo hermano  
Y luz alumbrando, la ruta del alma del que estoy amando...”  
Violeta Parra, 1966

Houve vezes que eu pensei não ser capaz de desenvolver uma tese de doutorado até o fim. Hoje vejo que consegui contribuir (seja qual for a dimensão) com os conhecimentos dos meus estudos para ciência. Foi difícil desempenhar o papel como ‘mãe’ dos meus pais e pós-graduanda ao mesmo tempo. Muitas vezes faltou energia e até mesmo duvidei da minha capacidade como pesquisadora. Ambos, pais e tese, têm prazo determinado. Considero que consegui cumprir os dois papéis. Tardei, mas não falhei (assim espero).

Houve pessoas que foram cruciais e contribuíram de alguma forma, grande ou pequena, científica ou emocional, para a realização desta tese. Para você que colaborou (conscientemente ou não, tanto da sua parte, como da minha) para a realização deste trabalho, a minha imensa gratidão e a bela canção de Violeta Parra. Todo ato de gentileza retorna para a nossa vida através de bênçãos, por isso sou grata.

Agradeço meu orientador, professor Gerhard Overbeck, por mais uma vez me orientar, mesmo extrapolando o número de orientados e aumentando ainda mais sua carga de trabalho. Obrigada por tudo! Desejo que toda a dedicação como professor retorne em forma de saúde, felicidades, espumantes, brindes, recursos para projetos, realizações e tudo de bom.

Agradeço o órgão financiador da bolsa de estudos, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao Programa de Pós-Graduação em Botânica da Universidade Federal do Rio Grande do Sul.

## SUMÁRIO

RESUMO .....	1
ABSTRACT .....	3
INTRODUÇÃO GERAL .....	5
Referências bibliográficas .....	9
CAPÍTULO 1: Seasonal patterns of endozoochory by cattle in subtropical grassland in southern Brazil .....	17
Acknowledgments .....	17
Abstract .....	18
Introduction .....	19
Materials and methods.....	21
Study area .....	21
Dung sampling and seedling emergence .....	23
Survey of vegetation.....	24
Data analysis .....	24
Results .....	25
Dung seed content .....	25
Relation between dung seed bank and vegetation.....	30
Discussion .....	31
Seasonal patterns of seed dispersal by cattle dung.....	31
Variation of functional characteristics of species transported by cattle dung in response to seasons and distinct grazing intensities .....	32
Relationship of the dung samples to availability of seed in the grassland community .....	35
Can seed transport in cattle dung contribute to grassland restoration? .....	36
References .....	37
Appendices .....	43
CAPÍTULO 2: Flowering and fruiting phenology patterns in South Brazilian subtropical grasslands from a restoration perspective .....	51
Abstract .....	52
Implications for practice.....	53
Introduction .....	54
Materials and methods.....	56
Study site .....	56
Phenological survey of vegetation .....	58
Data analysis .....	59
Results .....	60
Phenology characteristics of functional groups in relation to seasonality.....	60
Seasonal variation of flowering and fruiting in relation to grazing intensities .....	62

Discussion .....	64
Phenological patterns of subtropical grazed grasslands and implications for restoration .....	64
Phenological characteristics of functional groups in relation to seasonality .....	65
Seasonal variation of flowering and fruiting in relation to grazing intensities .....	66
Conclusions and perspectives.....	67
Acknowledgements .....	68
References .....	69
Supplementary material.....	77
CAPÍTULO 3: Seedling emergence from seed rain in subtropical grasslands under different grazing intensities.....	87
Abstract .....	88
Introduction .....	89
Material and methods .....	91
Study site and experimental design.....	91
Seed rain and seedling emergence.....	92
Vegetation surveys .....	93
Data analysis .....	93
Results .....	95
Seed trap content .....	95
Description of groups present in the seed traps.....	96
Relationship between seed traps records and established vegetation.....	99
Discussion .....	101
Seed rain in subtropical grasslands under distinct grazing intensities.....	101
Characterization of seed rain according to functional attributes .....	102
Similarity between seed rain and established vegetation .....	104
Acknowledgements .....	106
References .....	106
Supporting Information .....	115
CONSIDERAÇÕES FINAIS .....	137
ANEXOS.....	140
Outras atividades desenvolvidas durante o doutorado .....	140

## **RESUMO**

Diversos modelos teorizam a importância de considerar as variações espaciais e temporais para a análise da coexistência de espécies. Entretanto, quais são os fatores, mecanismos e processos responsáveis pela manutenção dessa riqueza? Em busca destas respostas, buscamos aprimorar a compreensão das estratégias e mecanismos relacionados à manutenção e regeneração da vegetação campestre, como: a dispersão ou chuva de sementes (provenientes da comunidade local e de áreas adjacentes), o banco de sementes do solo e transferência de sementes por ação antropogênica. A disponibilidade de propágulos é um dos principais fatores determinantes na montagem da comunidade vegetal. Muitas espécies com um banco de sementes do solo transitório dependem do suprimento constante de sementes dispersas para a regeneração de suas populações quando desaparecem da vegetação estabelecida. A endozoocoria pelo gado pode ser uma importante fonte de sementes nativas campestres, principalmente gramíneas, além de auxiliar na dispersão a longas distâncias e favorecer a germinação das sementes através da passagem pelo sistema digestório do animal e fornecimento de matéria orgânica. Quando os processos naturais não fornecem sementes suficientes para a recuperação das pastagens após a degradação, transporte de feno contendo sementes pode ser uma opção para superar a limitação de sementes na restauração ecológica. No entanto, sua aplicação requer o conhecimento acerca da floração e frutificação das espécies campestres para a coleta do feno com sementes maduras para transferência. Por fim, um melhor entendimento de parte das sementes que realmente entra no solo, para recrutamento direto ou para o banco de sementes do solo, também é um fator importante. Nesta tese, investigamos detalhadamente estes processos, tendo como base experimentos e levantamentos da vegetação, em campos subtropicais sob diferentes regimes de pastejo, incluindo exclusão de pastagem. Nos três estudos apresentados nos capítulos a seguir, nós observamos que a floração, a frutificação e a dispersão de sementes ocorrem, em nosso sistema de estudo, ao longo do ano inteiro, embora com uma clara variação na composição de

espécies (em termos taxonômicos e funcionais), em resposta à sazonalidade do clima e ao manejo da vegetação. Os resultados deste estudo contribuem para o entendimento da dinâmica da vegetação, podendo ser aplicados no manejo e restauração de pastagens.

**Palavras-chave:** fenologia, inflorescências, zoocoria, anemocoria, colonização, germinação, emergência de plântulas, intensidades de pastejo, diretrizes de restauração, Pampa.

## **ABSTRACT**

Several models contribute to our understanding of the importance of spatial and temporal variations in the analysis of species coexistence. However, what are the factors, mechanisms and processes responsible for maintaining the species richness of plant communities? In search of these answers, we aim to improve the understanding of the strategies and mechanisms related to the maintenance and regeneration of grassland vegetation, such as dispersal or seed rain (from the local community and adjacent areas) and by human-induced seed transfer. The availability of propagules is one of the driving factors for the establishment of the plant community. Many species with transient seed bank depend on the constant supply of dispersed seeds for the regeneration of their populations when they disappear from established vegetation. Endozoochory by cattle can be an important source of native grasslands seeds, mainly of grasses, in addition to assist in the dispersion over long distances and favor the germination of the seeds through the passage through the digestive system of the animal and through the supply of organic matter. Where natural processes do not bring sufficient seed for recovery of grasslands after degradation, the input of hay containing seeds may be an option for overcoming seed limitation in ecological restoration. However, its application requiring knowledge about flowering and fruiting of grassland species so that mature seed material will be transferred. Finally, a better understanding of that part of seeds that actually enters the soil, for direct recruitment or for the soil seed bank is also an important factor. In this thesis we investigate these processes in detail, based on experiments and observational surveys, in subtropical grasslands under different grazing regimes, including grazing exclusion. In three studies presented in the following chapters, we observed that flowering, fruiting and seed dispersal occur, in our study system, throughout the entire year, although with a clear variation in species composition (in taxonomic and functional terms) in response to seasonality of climate



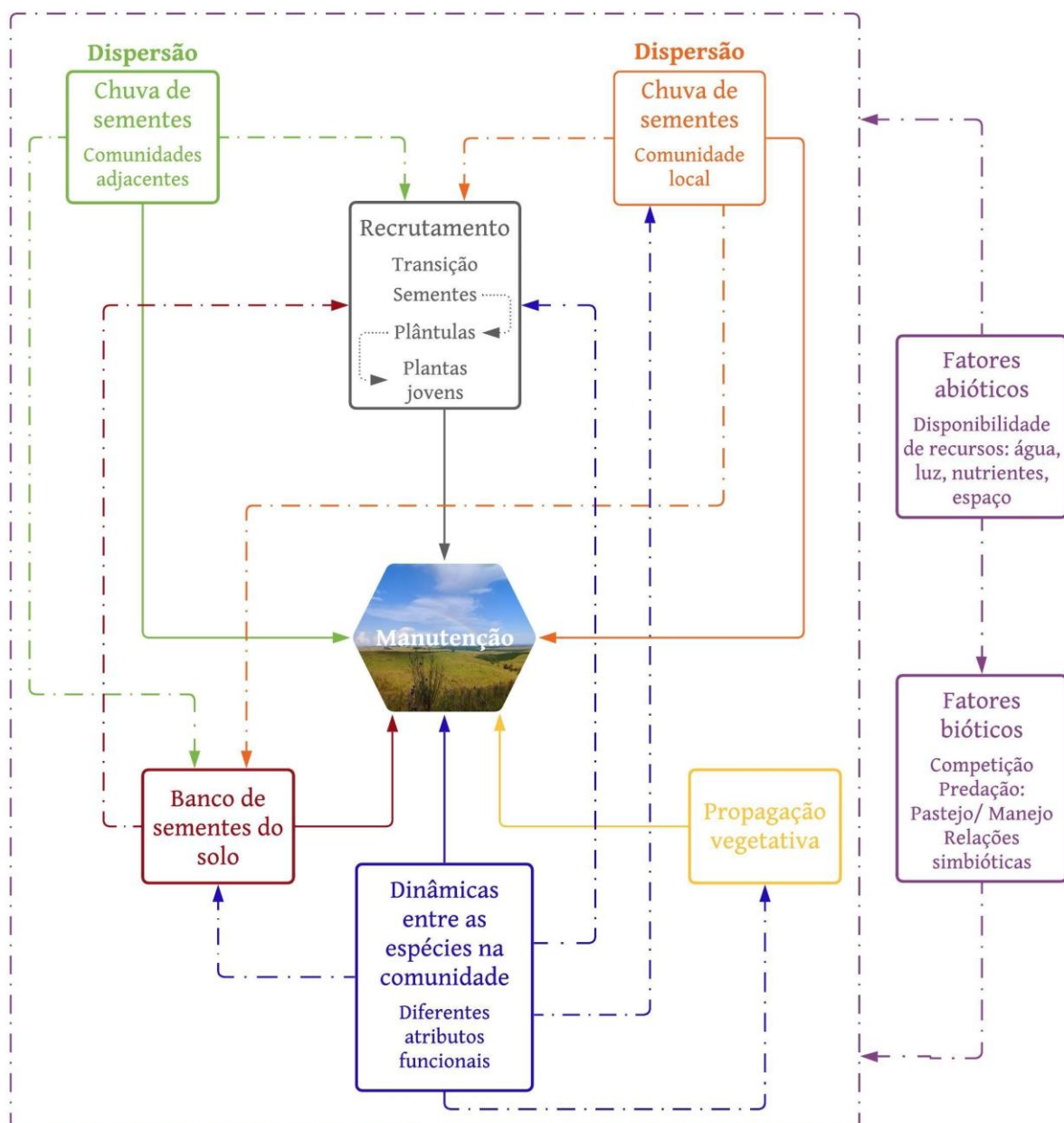
and vegetation management. The results of the study contribute to the understanding of vegetation dynamics, and can be applied in grassland management and restoration.

**Key words:** phenology, inflorescences, zoochory, anemochory, colonization, germination, seedling emergence, grazing intensities, restoration guidelines, Pampa.

## INTRODUÇÃO GERAL

Os ecossistemas campestres apresentam uma alta riqueza de espécies em diferentes escalas espaciais (Veldman et al., 2015; Wilson et al., 2012). Em apenas uma única parcela de 1 x 1 m foram registradas 56 espécies em estudo no Pampa brasileiro (da Silva Menezes et al., 2018) e 89 espécies em estudo feito na Argentina, sendo este o atual recorde mundial para a riqueza de espécies na escala de 1m<sup>2</sup> (Cantero et al., 1999). Estes são exemplos práticos que mostram a real dimensão da riqueza encontrada nos ecossistemas campestres, e diversos modelos têm sido propostos para explicar a alta diversidade e a coexistência de espécies. Entre eles, o *Modelo de Carrossel*, desenvolvido por Maarel and Sykes (1993), descreve a dinâmica de mobilidade das espécies (*turnover*) considerando tanto escalas temporais como espaciais. Segundo os autores, pequenos espaços de solo descoberto de vegetação, após o desaparecimento da planta por morte ou herbivoria, são suficientes para a colonização e estabelecimento de outras espécies, caracterizando um ciclo de substituição em torno de cada parte da comunidade, podendo ser um processo de rápida ou lenta duração. O *Modelo de Loteria*, desenvolvido por Sale (1977), afirma que as espécies situadas mais próximas a abertura de solo descoberto são as primeiras a colonizar este espaço, ou seja, aleatoriamente. Shmida and Ellner (1984) desenvolveram o *Modelo de Loteria* para comunidades vegetais, e além de demonstrarem que a competição por espaço é determinada ao acaso, constataram que não havia diferenciação nos nichos e que coexistência de espécies era possível pelas diferenças nos estágios de vida. Em suma, tais modelos teorizam a importância de considerar as variações espaciais e temporais para a análise de coexistência de espécies. Mas quais seriam os fatores e mecanismos responsáveis pela manutenção dessa riqueza? Como funcionam os processos ecológicos envolvidos na manutenção e regeneração da vegetação ao nível de populações? Como a expansão de espécies invasoras pode afetar a riqueza e composição nativa da comunidade? Em busca destas respostas, é necessário aprimorar a compreensão das estratégias

e mecanismos envolvidos na manutenção e regeneração da vegetação, assim como, o detalhamento dos fatores que influenciam a composição da comunidade campestre. A elaboração de um modelo conceitual (Fig. 1), destacando as etapas envolvidas nestes processos e fatores que os influenciam na manutenção campestre, foi o primeiro passo para organização dos tópicos aos quais buscamos respostas.



**Figura 1.** Modelo conceitual destacando os processos presentes e os fatores que influenciam na dinâmica de manutenção e regeneração da vegetação da comunidade campestre.

A regeneração e manutenção da vegetação campestre estão relacionadas à dispersão de propágulos ou chuva de sementes, provenientes da comunidade local e de áreas adjacentes, do banco de sementes do solo (BSS) (Gasparino et al., 2006) e ao rebrote e, em alguns casos, à propagação vegetativa, através da presença de *bud banks*, órgãos subterrâneos protegidos abaixo do solo (Overbeck et al., 2005; Fidelis and Blanco, 2014). Os fatores bióticos (competição, predação/pastejo, relações simbióticas) e os abióticos (disponibilidade de água, luz e nutrientes do solo) podem exercer influência na composição e estrutura da vegetação, tal como afetar as etapas do ciclo de regeneração e manutenção da vegetação da comunidade (Fig. 1).

A dinâmica, a organização e a própria manutenção dos campos estão associadas a fatores como o pastejo e o fogo (Andrade et al., 2019; Bond and Keeley, 2005). Estes processos atuam nas mudanças na riqueza, diversidade e dominância das espécies (Bazzaz, 1983; Haretche and Rodríguez, 2006), através dos efeitos sobre a competição interespecífica e transporte de propágulos (Milchunas et al., 1988), podendo modificar os processos de sucessão (Kinucan and Smeins, 1992). Estas informações vêm de encontro com o proposto pela hipótese do distúrbio intermediário (IDH), ao qual afirma que riqueza é maior em níveis moderados de perturbação (Grime, 1979), devido ao fato que nessa situação, não teremos uma dominância muito alta por poucas espécies que possuem o potencial de dominar a comunidade, assim diminuindo a riqueza por competição, nem perturbações tão frequentes que excluem muitas das espécies presentes (Overbeck et al., 2005). Entretanto, para a manutenção da riqueza e composição típica de espécies sob regimes de distúrbios é necessária a existência de um reservatório de propágulos. O reservatório de propágulos, já presente no solo ou disperso pela chuva de sementes, depende em grande parte da própria composição de espécies da área em questão, da configuração da paisagem, do manejo e do histórico de uso do solo (Müller et al., 2014). Espécies com um BSS persistente ou com a capacidade de dispersão de longa distância (por exemplo, por anemocoria

ou zoocoria) são mais bem-sucedidas no restabelecimento em curto prazo (Cosyns et al., 2005; von Blanckenhagen and Poschlod, 2005). No entanto, o BSS transitório é composto por espécies presentes na vegetação estabelecida e na camada superior do solo (Thompson et al., 1997) ou por sementes que permanecem no solo por menos de ano após a dispersão dos propágulos (Baker, 1989). Portanto, as espécies com BSS transitório dependem do suprimento constante de sementes dispersas (ou seja, a chuva de sementes) de áreas adjacentes para a regeneração das suas populações quando desaparecem da vegetação estabelecida (Marone et al., 1998). A disponibilidade de propágulos e, em menor medida, a disponibilidade de espaços adequados para a germinação, são bem conhecidos como fatores determinantes na montagem da comunidade de plantas (Marteinsdóttir, 2014; Müller et al., 2014). O conhecimento dos processos de dispersão de sementes é importante para avaliar o papel relativo desses processos.

Os biomas campestres estão entre os ecossistemas naturais mais ameaçados do mundo, principalmente pela transformação em outros tipos de uso, invasão de espécies exóticas e regimes de manejo inadequado, por exemplo, o sobrepastejo (Buisson et al., 2019). Os recentes avanços nas pesquisas ecológicas e relacionadas à restauração ecológica são motivados por estas ameaças e acompanhados pela crescente conscientização das consequências da degradação ambiental. Para se traçar as estratégias de um projeto de restauração e do manejo adequado em áreas degradadas, o entendimento da dinâmica da vegetação e dos processos envolvidos da sua regeneração natural é fundamental.

Com o intuito de fornecer informações detalhadas sobre os mecanismos responsáveis pela manutenção da vegetação campestre, tema de alta relevância para o manejo da vegetação nativa e a recuperação de áreas degradadas, foram desenvolvidos os artigos apresentados nos seguintes capítulos:

- **Capítulo 1:** Seasonal patterns of endozoochory by cattle in subtropical grassland in southern Brazil. O manuscrito foi apresentado no exame de qualificação está publicado online na revista *Austral Ecology* (<https://onlinelibrary.wiley.com/doi/10.1111/aec.13054>);

- **Capítulo 2:** Flowering and fruiting phenology patterns in South Brazilian subtropical grasslands from a restoration perspective. O manuscrito será submetido à revista *Restoration Ecology*;

- **Capítulo 3:** Seedling emergence from seed rain in subtropical grasslands under different grazing intensities. O manuscrito será submetido à revista *Rangeland Ecology & Management*.

Para complementar os tópicos acerca dos mecanismos de manutenção e regeneração campestre, mas não presentes na tese, um estudo de revisão sobre banco de sementes do solo está em desenvolvimento em cooperação com a pesquisadora Patrícia Carla de Oliveira (Universidade Federal de Mato Grosso). Nosso objetivo é explanar o conhecimento atual sobre banco de sementes do solo em campos naturais tropicais e subtropicais, campos temperados e savanas com predomínio de gramíneas C4, com o intuito de compreender se há tendências, pontos e mecanismos em comum dentro e entre estes ecossistemas, bem como estes resultados podem contribuir em estudos e projetos de restauração de ecossistemas campestres degradados. Adicionalmente, também ainda está em processo de compilação, um estudo testando a eficácia entre diferentes armadilhas de sementes (armadilha pegajosa, em funil e potes de substrato estéril) em nosso sistema do estudo, visando compreender melhor lacunas não totalmente compreendidas na análise da chuva de sementes.

### **Referências bibliográficas**

- Adler, P., Raff, D., Lauenroth, W., 2001. The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*. <https://doi.org/10.1007/s004420100737>
- Agostinelli, C., Lund, U., 2011. R package circular: Circular Statistics (version 0.4-3). CA Dep. Environ. Sci. Informatics Stat. Ca' Foscari Univ. Venice, Italy. UL Dep. Stat. Calif. Polytech. State Univ. San Luis Obispo, California, USA.
- Albert, Á.J., Mudrák, O., Jongepierová, I., Fajmon, K., Frei, I., Ševčíková, M., Klimešová, J.,

- Doležal, J., 2019. Grassland restoration on ex-arable land by transfer of brush-harvested propagules and green hay. *Agric. Ecosyst. Environ.* 272, 74–82.  
<https://doi.org/10.1016/j.agee.2018.11.008>
- Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. *Meteorol. Zeitschrift* 22, 711–728.  
<https://doi.org/10.1127/0941-2948/2013/0507>
- Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecol. Monogr.*  
<https://doi.org/10.1890/12-2010.1>
- Andrade, B.O., Bonilha, C.L., Overbeck, G.E., Vélez-Martin, E., Rolim, R.G., Bordignon, S.A.L., Schneider, A.A., Vogel Ely, C., Lucas, D.B., Garcia, É.N., dos Santos, E.D., Torchelsen, F.P., Vieira, M.S., Silva Filho, P.J.S., Ferreira, P.M.A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V.D., Boldrini, I.I., 2019. Classification of South Brazilian grasslands: Implications for conservation. *Appl. Veg. Sci.* 22, 168–184.  
<https://doi.org/10.1111/avsc.12413>
- Arruda, A.J., Buisson, E., Poschlod, P., Silveira, F.A.O., 2018. How have we studied seed rain in grasslands and what do we need to improve for better restoration? *Restor. Ecol.*  
<https://doi.org/10.1111/rec.12686>
- Baker, H.G., 1989. Some Aspects of the Natural History of Seed Banks, in: *Ecology of Soil Seed Banks*. pp. 9–21. <https://doi.org/10.1016/b978-0-12-440405-2.50007-5>
- Bazzaz, F.A., 1983. Characteristics of populations in relation to disturbance in natural and man-modified ecosystems., in: Mooney, H.A., Godron, M. (Eds.), *Disturbance and Ecosystems*. Berlin, pp. 259–275. [https://doi.org/10.1007/978-3-642-69137-9\\_17](https://doi.org/10.1007/978-3-642-69137-9_17)
- Benke, C.S.C., Morellato, L.P.C., 2002. Comparação de dois métodos de avaliação da fenologia de plantas, sua interpretação e representação. *Rev. Bras. Botânica* 25, 269–275. <https://doi.org/10.1590/s0100-84042002000300003>
- Beretta, M.E., Fernandes, A.C., Schneider, A.A., Ritter, M.R., 2008. A família Asteraceae no Parque Estadual de Itapuã, Viamão, Rio Grande do Sul, Brasil. *Rev. Bras. Biociência* 6, 189–216.
- Bergmeier, E., Matthäs, U., 1996. Quantitative studies of phenology and early effects of non-grazing in Cretan phrygana vegetation. *J. Veg. Sci.* 7, 229–236.  
<https://doi.org/10.2307/3236323>
- Bischoff, A., Hoboy, S., Winter, N., Warthemann, G., 2018. Hay and seed transfer to re-establish rare grassland species and communities: How important are date and soil preparation? *Biol. Conserv.* 221, 182–189.  
<https://doi.org/https://doi.org/10.1016/j.biocon.2018.02.033>
- Boavista, L. da R., Trindade, J.P.P., Overbeck, G.E., Müller, S.C., 2019. Effects of grazing regimes on the temporal dynamics of grassland communities. *Appl. Veg. Sci.* 22, 326–335. <https://doi.org/10.1111/avsc.12432>
- Boldrini, I.I., Longhi-Wagner, H.M., Boechat, S. de C., 2008. *Morfologia e taxonomia de gramíneas Sul-Rio-Grandenses*, 2 nd. ed. UFRGS Editora, Porto Alegre.
- Bond, W.J., Keeley, J.E., 2005. Fire as a global “herbivore”: The ecology and evolution of flammable ecosystems. *Trends Ecol. Evol.* 20, 387–394.  
<https://doi.org/10.1016/j.tree.2005.04.025>
- Buisson, E., Alvarado, S.T., Le Stradic, S., Morellato, L.P.C., 2017. Plant phenological

- research enhances ecological restoration. *Restor. Ecol.* 25, 164–171.  
<https://doi.org/10.1111/rec.12471>
- Buisson, E., Le Stradic, S., Silveira, F.A.O., Durigan, G., Overbeck, G.E., Fidelis, A., Fernandes, G.W., Bond, W.J., Hermann, J.-M., Mahy, G., Alvarado, S.T., Zaloumis, N.P., Veldman, J.W., 2019. Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. *Biol. Rev.* 94, 590–609.  
<https://doi.org/10.1111/brv.12470>
- Bullock, J.M., Moy, I.L., 2004. Plants as seed traps: Inter-specific interference with dispersal. *Acta Oecologica*. <https://doi.org/10.1016/j.actao.2003.10.005>
- Burkart, A.E., 1969. *Flora ilustrada de Entre Rios (Argentina)*, Instituto Nacional de Tecnología Agropecuaria. Buenos Aires.
- Campbell, C.S., 1985. The subfamilies and tribes of Gramineae (Poaceae) in the southeastern United States. *J. Arnold Arboretum*. 66, 123–199. <https://doi.org/10.5962/bhl.part.13181>
- Cantero, J.J., Partel, M., Zobel, M., 1999. Is Species Richness Dependent on the Neighbouring Stands? An Analysis of the Community Patterns in Mountain Grasslands of Central Argentina. *Oikos* 87, 346–354. <https://doi.org/10.2307/3546750>
- Chabrierie, O., Alard, D., 2005. Comparison of three seed trap types in a chalk grassland: Toward a standardised protocol. *Plant Ecol.* <https://doi.org/10.1007/s11258-004-0024-2>
- Clinebell, R.R., 2005. Tips for gathering individual species, in: Stephen Packard, C.F.M. (Ed.), *The Tallgrass Restoration Handbook*. Island press, pp. 127–134.
- Coiffait-Gombault, C., Buisson, E., Dutoit, T., 2011. Hay Transfer Promotes Establishment of Mediterranean Steppe Vegetation on Soil Disturbed by Pipeline Construction. *Restor. Ecol.* 19, 214–222. <https://doi.org/10.1111/j.1526-100X.2010.00706.x>
- Cosyns, E., Claerbout, S., Lamoot, I., Hoffmann, M., 2005. Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecol.* 178, 149–162.  
<https://doi.org/10.1007/s11258-004-2846-3>
- Cruz, P., De Quadros, F.L.F., Theau, J.P., Frizzo, A., Jouany, C., Duru, M., Carvalho, P.C.F., 2010. Leaf traits as functional descriptors of the intensity of continuous grazing in native grasslands in the South of Brazil. *Rangel. Ecol. Manag.* 63, 350–358.  
<https://doi.org/10.2111/08-016.1>
- da Silva Menezes, L., Vogel Ely, C., Lucas, D.B., Minervini Silva, G.H., Boldrini, I.I., Overbeck, G.E., 2018. Plant species richness record in Brazilian Pampa grasslands and implications. *Rev. Bras. Bot.* 41, 817–823. <https://doi.org/10.1007/s40415-018-0492-6>
- de Souza Vieira, M., Overbeck, G.E., 2020. Small seed bank in grasslands and tree plantations in former grassland sites in the South Brazilian highlands. *Biotropica*. <https://doi.org/10.1111/btp.12785>
- Deble, L.P., Marchiori, J.N.C., 2014. Sinopse do gênero *Gamochaeta* Weddel (Asteraceae-Gnaphalieae) no Brasil. *Balduinia* 0, 21–31. <https://doi.org/10.5902/2358198014040>
- Díaz, S., Acosta, A., Cabido, M., Diaz, S., 1994. Grazing and the Phenology of Flowering and Fruiting in a Montane Grassland in Argentina: A Niche Approach. *Oikos* 70, 287.  
<https://doi.org/10.2307/3545640>
- Duarte, E., Aona-Pinheiro, Lidyanne Costa, G., Bezerra, C., Soares de Oliveira Neto, J., 2019. Glossário eletrônico de morfologia vegetal: sementes e propágulos [WWW Document]. Univ. Fed. Góias/ Univ. Fed. do Recôncavo da Bahia. URL [www.projetosbotanica.icb.ufg.br/guia\\_sd/glossary](http://www.projetosbotanica.icb.ufg.br/guia_sd/glossary) (accessed 11.18.20).



- Fedrigo, J.K., Ataíde, P.F., Filho, J.A., Oliveira, L. V., Jaurena, M., Laca, E.A., Overbeck, G.E., Nabinger, C., 2018. Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. *Restor. Ecol.* 26, 677–685. <https://doi.org/10.1111/rec.12635>
- Feldman, S.R., Alzugaray, C., Lewis, J.P., 2007. Relación entre la vegetación y el banco de semillas de un espartillar de *Spartina argentinensis*. *Cienc. e Investig. Agrar.* 34, 41–48. <https://doi.org/10.4067/s0718-16202007000100005>
- Ferreira, P.M.A., Andrade, B.O., Podgaiski, L.R., Dias, A.C., Pillar, V.D., Overbeck, G.E., Milton de, S.M., Boldrini, I.I., 2020. Long-term ecological research in southern Brazil grasslands: Effects of grazing exclusion and deferred grazing on plant and arthropod communities. *PLoS One* 15, 1–23. <https://doi.org/10.1371/journal.pone.0227706>
- Fetzel, T., Havlik, P., Herrero, M., Erb, K.H., 2017. Seasonality constraints to livestock grazing intensity. *Glob. Chang. Biol.* 23, 1636–1647. <https://doi.org/10.1111/gcb.13591>
- Fidelis, A., Blanco, C., 2014. Does fire induce flowering in Brazilian subtropical grasslands? *Appl. Veg. Sci.* 17, 690–699. <https://doi.org/10.1111/avsc.12098>
- Fischer, F.M., Bonnet, O.J.F., Cezimbra, I.M., Pillar, V.D., 2019. Long-term effects of grazing intensity on strategies and spatial components of functional diversity in subtropical grassland. *Appl. Veg. Sci.* 22, 39–47. <https://doi.org/10.1111/avsc.12402>
- Flora do Brasil 2020, 2020. Flora do Brasil 2020 [WWW Document]. Jard. Botânico do Rio Janeiro. URL [www.floradobrasil.jbrj.gov.br](http://www.floradobrasil.jbrj.gov.br) (accessed 11.12.20).
- Funes, G., Basconcelo, S., Díaz, S., Cabido, M., 2001. Edaphic patchiness influences grassland regeneration from the soil seed-bank in mountain grasslands of central Argentina. *Austral Ecol.* 26, 205–212. <https://doi.org/10.1046/j.1442-9993.2001.01102.x>
- Galíndez, G., Ortega-Baes, P., Scopel, A.L., Hutchings, M.J., 2013. The dynamics of three shrub species in a fire-prone temperate savanna: The interplay between the seed bank, seed rain and fire regime. *Plant Ecol.* 214, 75–86. <https://doi.org/10.1007/s11258-012-0147-9>
- Gasparino, D., Malavasi, U.C., Malavasi, M. de M., Souza, I. de, 2006. Quantificação do banco de sementes sob diferentes usos do solo em área de domínio ciliar. *Rev. Árvore* 30, 1–9. <https://doi.org/10.1590/s0100-67622006000100001>
- Gonnet, J.M., 2001. Influence of cattle grazing on population density and species richness of granivorous birds (Emberizidae) in the arid plain of the Monte, Argentina. *J. Arid Environ.* 48, 569–579. <https://doi.org/10.1006/jare.2000.0777>
- Grime, J.P., 1979. *Plant strategies and vegetation process*, 1 st. ed. John Wiley and Sons, Chichester.
- Han, J., Chen, J., Xia, J., Li, L., 2015. Grazing and watering alter plant phenological processes in a desert steppe community. *Plant Ecol.* 216, 599–613. <https://doi.org/10.1007/s11258-015-0462-z>
- Haretche, F., Rodríguez, C., 2006. Banco de semillas de un pastizal uruguayo bajo diferentes condiciones de pastoreo. *Ecol. Austral* 16, 105–113.
- Janzen, D.H., 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *Am. Nat.* <https://doi.org/10.1086/284208>
- Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L., Hölzel, N., 2010. Species introduction in restoration projects - Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic Appl. Ecol.* 11, 285–299.

- <https://doi.org/10.1016/j.baae.2009.12.004>
- Kiehl, K., Wagner, C., 2006. Effect of Hay Transfer on Long-Term Establishment of Vegetation and Grasshoppers on Former Arable Fields. *Restor. Ecol.* 14, 157–166. <https://doi.org/10.1111/j.1526-100X.2006.00116.x>
- Kinucan, R.J., Smeins, F.E., 1992. Soil Seed Bank of a Semiarid Texas Grassland Under Three Long-Term (36- Years) Grazing Regimes. *Am. Midl. Nat.* 128, 11–21. <https://doi.org/10.2307/2426408>
- Kolb, R.M., Pilon, N.A.L., Durigan, G., 2016. Factors influencing seed germination in Cerrado grasses . *Acta Bot. Bras.* .
- Kollmann, J., Goetze, D., 1998. Notes on seed traps in terrestrial plant communities. *Flora* 193, 31–40. [https://doi.org/10.1016/S0367-2530\(17\)30813-7](https://doi.org/10.1016/S0367-2530(17)30813-7)
- Le Stradic, S., Buisson, E., Fernandes, G.W., 2014. Restoration of Neotropical grasslands degraded by quarrying using hay transfer. *Appl. Veg. Sci.* 17, 482–492. <https://doi.org/10.1111/avsc.12074>
- Le Stradic, S., Buisson, E., Fernandes, G.W., Morellato, L.P.C., 2018. Reproductive phenology of two co-occurring Neotropical mountain grasslands. *J. Veg. Sci.* 29, 15–24. <https://doi.org/10.1111/jvs.12596>
- Lemon, A.J., Bolker, B., Oom, S., Klein, E., Rowlingson, B., Wickham, H., Tyagi, A., Eterradosi, O., Grothendieck, G., Toews, M., Kane, J., Turner, R., Witthoft, C., Stander, J., Petzoldt, T., Duursma, R., Biancotto, E., Levy, O., Dutang, C., Solymos, P., Engelmann, R., Hecker, M., Steinbeck, F., Borchers, H., Singmann, H., Toal, T., Ogle, D., Lemon, M.J., 2016. Package ‘plotrix.’
- Lennartsson, T., Wissman, J., Bergström, H.M., 2012. The effect of timing of grassland management on plant reproduction. *Int. J. Ecol.* 2012. <https://doi.org/10.1155/2012/156274>
- Leon, R.J.C., Bertiller, M., 1982. Aspectos fenológicos de dos comunidades del pastizal de la Depresion del Salado. *Bol. la Soc. Argentina Bot.* 20, 329–348.
- Lezama, F., Baeza, S., Altesor, A., Cesa, A., Chaneton, E.J., Paruelo, J.M., 2014. Variation of grazing-induced vegetation changes across a large-scale productivity gradient. *J. Veg. Sci.* <https://doi.org/10.1111/jvs.12053>
- Li, Yu, Dong, S., Gao, Q., Zhang, Y., Liu, S., Swift, D., Ganjurjav, H., Hu, G., Wang, X., Yan, Y., Wu, H., Luo, W., Ge, Y., Li, Yan, Zhao, Z., Gao, X., Li, S., Song, J., 2019. The effects of grazing regimes on phenological stages, intervals and divergences of alpine plants on the Qinghai-Tibetan Plateau. *J. Veg. Sci.* 30, 134–145. <https://doi.org/10.1111/jvs.12703>
- Linabury, M.C., Turley, N.E., Brudvig, L.A., 2019. Insects remove more seeds than mammals in first-year prairie restorations. *Restor. Ecol.* <https://doi.org/10.1111/rec.13004>
- Londo, G., 1976. The decimal scale for relevés of permanent quadrats. *Vegetatio*. <https://doi.org/10.1007/BF00055300>
- Loydi, A., Zalba, S.M., Distel, R.A., 2012. Viable seed banks under grazing and enclosure conditions in montane mesic grasslands of Argentina. *Acta Oecologica* 43, 8–15. <https://doi.org/10.1016/j.actao.2012.05.002>
- Lucero, J.E., Callaway, R.M., 2018. Granivory from native rodents and competition from an exotic invader strongly and equally limit the establishment of native grasses. *Oecologia*. <https://doi.org/10.1007/s00442-018-4085-7>

- Maarel, E., Sykes, M.T., 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *J. Veg. Sci.* 4, 179–188. <https://doi.org/10.2307/3236103>
- Marone, L., Rossi, B.E., De Casenave, J.L., 1998. Granivore impact on soil-seed reserves in the central Monte desert, Argentina. *Funct. Ecol.* 12, 640–645. <https://doi.org/10.1046/j.1365-2435.1998.00226.x>
- Márquez, S., Funes, G., Cabido, M., Pucheta, E., Multidisciplinario, I., Vegetal, D.B., Cátedra, I., Geofísica, D. De, Biología, A., Exactas, F.D.C., Naturales, F., Nacional, U., Juan, D.S., Juan-argentina, S., 2002. Efectos del pastoreo sobre el banco de semillas germinable y la vegetación establecida en pastizales de montaña del centro de Argentina. *Grazing effects on the germinable seed bank and standing vegetation in mountain grasslands from central Argentina* El pa 327–337.
- Marteinsdóttir, B., 2014. Seed rain and seed bank reveal that seed limitation strongly influences plant community assembly in grasslands. *PLoS One* 9, e103352. <https://doi.org/10.1371/journal.pone.0103352>
- Martínková, J., Smilauer, P., Mihulka, S., 2002. Phenological pattern of grassland species: relation to the ecological and morphological traits. *Flora - Morphol. Distrib. Funct. Ecol. Plants* 197, 290–302. <https://doi.org/https://doi.org/10.1078/0367-2530-00043>
- Milchunas, D.G., Sala, O.E., Lauenroth, W.K., 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* 132, 87–106. <https://doi.org/10.1086/284839>
- Minervini-Silva, G.H., Overbeck, G.E., 2020. Soil seed bank in a subtropical grassland under different grazing intensities. *Acta Bot. Brasilica* 34, 360–370. <https://doi.org/10.1590/0102-33062019abb0297>
- Minervini Silva, G.H., Overbeck, G.E., 2021. Seasonal patterns of endozoochory by cattle in subtropical grassland in southern Brazil. *Austral Ecol. aec.13054*. <https://doi.org/10.1111/aec.13054>
- Morellato, L.P.C., Alberti, L.F., Hudson, I.L., 2010. Applications of circular statistics in plant phenology: A case studies approach, in: *Phenological Research: Methods for Environmental and Climate Change Analysis*. Springer Netherlands, pp. 339–359. [https://doi.org/10.1007/978-90-481-3335-2\\_16](https://doi.org/10.1007/978-90-481-3335-2_16)
- Müller, J., Heinze, J., Joshi, J., Boch, S., Klaus, V.H., Fischer, M., Prati, D., 2014. Influence of experimental soil disturbances on the diversity of plants in agricultural grasslands. *J. Plant Ecol.* 7, 509–517. <https://doi.org/10.1093/jpe/rtt062>
- Nabinger, C., Ferreira, E.T., Freitas, A.K., Carvalho, P.C.F., Sant’Anna, D.M., 2009. Produção animal com base no campo nativo: aplicações de resultados de pesquisa., in: *Campos Sulinos: Conservação e Uso Sustentável Da Biodiversidade*.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan: Community Ecology Package*. R package version 2.4-2. *Community Ecol. Packag.*
- Oleques, S.S., Overbeck, G.E., de Avia, R.S., 2017. Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. *Flora Morphol. Distrib. Funct. Ecol. Plants* 229, 141–146. <https://doi.org/10.1016/j.flora.2017.02.024>
- Oleques, S.S., Vizin-Bugoni, J., Overbeck, G.E., 2019. Influence of grazing intensity on patterns and structuring processes in plant–pollinator networks in a subtropical

- grassland. *Arthropod. Plant. Interact.* 13, 757–770. <https://doi.org/10.1007/s11829-019-09699-8>
- Overbeck, G.E., Hermann, J.M., Andrade, B.O., Boldrini, I.I., Kiehl, K., Kirmer, A., Koch, C., Kollmann, J., Meyer, S.T., Müller, S.C., Nabinger, C., Pilger, G.E., Trindade, J.P.P., Vélez-Martin, E., Walker, E.A., Zimmermann, D.G., Pillar, V.D., 2013. Restoration ecology in Brazil-time to step out of the forest. *Nat. a Conserv.* 11, 92–95. <https://doi.org/10.4322/natcon.2013.015>
- Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C., Boldrini, I.I., Both, R., Forneck, E., 2007. Brazil's neglected biome: The South Brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* 9, 101–116. <https://doi.org/10.1016/j.ppees.2007.07.005>
- Overbeck, G.E., Müller, S.C., Pillar, V.D., Pfadenhauer, J., 2005. Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *J. Veg. Sci.* 16, 655–664. <https://doi.org/10.1111/j.1654-1103.2005.tb02408.x>
- Palma, A.C., Laurance, S.G.W., 2015. A review of the use of direct seeding and seedling plantings in restoration: What do we know and where should we go? *Appl. Veg. Sci.* 18, 561–568. <https://doi.org/10.1111/avsc.12173>
- Pasini, E., Katinas, L., Ritter, M.R., 2014. O gênero *Chaptalia* ( Asteraceae , Mutisieae ) no Rio Grande do Sul , Brasil. *Rodriguésia* 65, 141–158.
- Peters, D.P.C., 2000. Climatic variation and simulated patterns in seedling establishment of two dominant grasses at a semi-arid-arid grassland ecotone. *J. Veg. Sci.* <https://doi.org/10.2307/3246579>
- Pillar, V.D.P., 2006. User s Guide v. 2.4. Memory.
- Pinheiro, M., Abrão, B.E. de, Harter-Marques, B., Miotto, S.T.S., 2008. Floral resources used by insects in a grassland community in Southern Brazil. *Rev. Bras. Botânica* 31. <https://doi.org/10.1590/s0100-84042008000300011>
- R Core Team (2020), 2020. R: A language and environment for statistical computing. R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. Vienna, Austria.
- R Core Team 2019, 2019. : A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL <http://www.R-project.org/>.
- Roberts, H.A., 1981. Seed banks in soils. *Adv. Appl. Biol.*
- Sale, P.F., 1977. Maintenance of High Diversity in Coral Reef Fish Communities. *Am. Nat.* 111, 337–359. <https://doi.org/10.1086/283164>
- Shmida, A., Ellner, S., 1984. Coexistence of plant species with similar niches. *Vegetatio* 58, 29–55. <https://doi.org/10.1007/BF00044894>
- Silva, I.A., Guido, A., Müller, S.C., 2020. Predicting plant performance for the ecological restoration of grasslands: the role of regenerative traits. *Restor. Ecol.* <https://doi.org/10.1111/rec.13182>
- Stucky, B.J., Guralnick, R., Deck, J., Denny, E.G., Bolmgren, K., Walls, R., 2018. The plant phenology ontology: A new informatics resource for large-scale integration of plant phenology data. *Front. Plant Sci.* 9, 1–12. <https://doi.org/10.3389/fpls.2018.00517>
- Thomas, P., Overbeck, G., Müller, S., 2019. Restoration of abandoned subtropical highland grasslands in Brazil: mowing produces fast effects, but hay transfer does not, *Acta Botanica Brasilica*. <https://doi.org/10.1590/0102-33062018abb0377>
- Thompson, K., Bakker, J.P., Bekker, R.M., 1997. Seed bank classification, in: *The Soil Seed*

- Banks of North West Europe: Methodology, Density and Longevity. Cambridge University Press, Melbourne, p. 276.
- Török, P., Migléc, T., Valkó, O., Kelemen, A., Tóth, K., Lengyel, S., Tóthmérész, B., 2012. Fast restoration of grassland vegetation by a combination of seed mixture sowing and low-diversity hay transfer. *Ecol. Eng.* 44, 133–138. <https://doi.org/10.1016/j.ecoleng.2012.03.010>
- Veldman, J.W., Buisson, E., Durigan, G., Fernandes, G.W., Le Stradic, S., Mahy, G., Negreiros, D., Overbeck, G.E., Veldman, R.G., Zaloumis, N.P., Putz, F.E., Bond, W.J., 2015. Toward an old-growth concept for grasslands, savannas, and woodlands. *Front. Ecol. Environ.* 13, 154–162. <https://doi.org/10.1890/140270>
- Vieira, M. de S., Bonilha, C.L., Boldrini, I.I., Overbeck, G.E., 2015. The seed bank of subtropical grasslands with contrasting land-use history in southern Brazil. *Acta Bot. Brasilica* 29, 543–552. <https://doi.org/10.1590/0102-33062015abb0026>
- Vogel Ely, C., 2019. Sistemática e conservação das espécies sul-americanas não andinas de *Hypericum* L. (HYPERICACEAE). *Fed. Univ. Rio Gd. do Sul. Federal University of Rio Grande do Sul.*
- von Blanckenhagen, B., Poschlod, P., 2005. Restoration of calcareous grasslands: The role of the soil seed bank and seed dispersal for recolonisation processes. *Biotechnol. Agron. Soc. Environ.* BASE 9, 143–149.
- Wagner, M., Hulmes, S., Hulmes, L., Redhead, J.W., Nowakowski, M., Pywell, R.F., 2020. Green hay transfer for grassland restoration: species capture and establishment. *Restor. Ecol.* 1–11. <https://doi.org/10.1111/rec.13259>
- Wilson, J.B., Peet, R.K., Dengler, J., Pärtel, M., 2012. Plant species richness: The world records. *J. Veg. Sci.* 23, 796–802. <https://doi.org/10.1111/j.1654-1103.2012.01400.x>
- Zar, J., 2010. *Biostatistical analysis*. 5th, Pearson Prentice-Hall, Upper Saddle River.
- Zarco, A., Cueto, V.R., Sagario, M.C., Marone, L., 2019. Effects of livestock grazing on flocks of seed-eating birds in the central monte desert, Argentina. *Can. J. Zool.* <https://doi.org/10.1139/cjz-2018-0223>
- Zhu, J., Zhang, Y., Liu, Y., 2016. Effects of short-term grazing exclusion on plant phenology and reproductive succession in a Tibetan alpine meadow. *Sci. Rep.* 6, 2–10. <https://doi.org/10.1038/srep27781>
- Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. *Analyzing Ecological Data, Methods*. <https://doi.org/10.1016/B978-0-12-387667-6.00013-0>

## **CAPÍTULO 1: Seasonal patterns of endozoochory by cattle in subtropical grassland in southern Brazil<sup>1</sup>**

Graziela Har Minervini Silva<sup>1</sup> <https://orcid.org/0000-0003-3835-7753> and

Gerhard Ernst Overbeck<sup>12</sup> <https://orcid.org/0000-0002-8716-5136>

<sup>1</sup>Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Botânica. Avenida Bento Gonçalves 9500, Prédio 43432, Sala 109, Porto Alegre, RS, Brazil.

E-mail: [graziela.minervini@gmail.com](mailto:graziela.minervini@gmail.com) Telefone: +55 51 3308 7559

<sup>2</sup>Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Botânica. Avenida Bento Gonçalves 9500, Porto Alegre, RS, Brazil.

### **Acknowledgments**

*Our special thanks go to the UFRGS Grazing Ecology Research Group, for maintaining the forage allowance experiment at UFRGS Agronomic Experimental Station for many years. We thank Miguel Dall'Agnol for offering space in the greenhouse of the Department of Agrometeorology and Forage Plants, UFRGS. For assistance in field and greenhouse work and in plant identification, we thank members of the Laboratory for the Study of Grassland Vegetation (Levcamp), UFRGS, especially Lucas Pinheiro, Luciana Menezes, Kassiane Helmicki, Helena Streit, Pedro Silva Filho and Ilsi Boldrini. We thank Ilsi Boldrini, Anaclara Guido, Danilo Sant'Anna and two anonymous reviewers for their helpful comments on the manuscript. This study was financed in part by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) research grant to GEO (477618/2013-8) and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. GEO acknowledges CNPq productivity grants (310022/2015-0 and 310345/2018-9) and GHMS CAPES scholarship.*

---

<sup>1</sup> Manuscrito apresentado no exame de qualificação e publicado online na revista *Austral Ecology* (<https://onlinelibrary.wiley.com/doi/10.1111/aec.13054>).

## **Abstract**

Seed dispersal influences plant community dynamics and regeneration. Zoochory by cattle is a particularly important type of dispersal in grasslands, but still poorly understood in subtropical grasslands. We studied endozoochory in a native grassland area with different grazing intensities, defined by distinct forage allowance levels. On one day in each of the four seasons of the year, dung samples were collected and kept in a greenhouse for germination over one full year. At each dung collection date, a survey of reproducing plants was conducted to relate the species composition of seed within dung to the community in dispersal phase. We used randomization tests to compare grazing intensities and seasons in terms of dung community richness, abundance and composition. We recorded 93 species in the dung samples, with 65 species in the spring samples, 61 in summer samples, 45 in the autumn samples and 37 in the winter samples. Of the 90 species identified in the vegetation, almost 50% (43 species) also occurred in dung samples. Graminoid herbs presented the largest number of species in all samples. Grazing had no effect on overall seed and species number, but was related to compositional differences in the cattle dung communities. Grazing also influenced the species composition in relation to life cycle, by the increase of annual species at the extremes of our grazing gradient (very high or very low grazing intensities). We conclude that endozoochory by cattle is an important process for the dispersal of native species in the studied system, with potential applications in grassland management and restoration. A better understanding of the strategies involved in seed dispersal is essential to improve knowledge about grassland vegetation regeneration and maintenance.

**KEYWORDS:** community dynamics, dispersal, ecological restoration, grazing, Pampa, seasonality, seeds, zoochory.

## Introduction

Cattle grazing is one of the main determinants of vegetation structure and composition of grasslands, principally due to the – often selective – consumption of above-growth plant biomass by grazing animals, but also due to other factors, such as soil compaction and animal trampling (Kinucan & Smeins 1992; Gasparino *et al.* 2006). On the other hand, vegetation structure, such as sward height and percentage of tussock grasses, also influences cattle behavior and thus grazing activity (Da Trindade *et al.* 2012). Further, different grazing intensities can promote evident changes in vegetation composition in terms of species and of functional groups (Cruz *et al.* 2010). At moderate grazing intensity, the vegetation shows a heterogeneous structure, composed of plants with contrasting habit (Adler *et al.* 2001) and, consequently, a higher diversity and richness of plant species (Loydi 2019). High grazing intensity sites are characterized by dominance of stoloniferous and/or rhizomatous grasses and herbs (Adler *et al.* 2001). In contrast, under low grazing intensity the preference of cattle for low-growing and more palatable grasses creates a positive feedback mechanism that ensures the dominance of tussock grasses and subshrubs (Cruz *et al.* 2010).

In addition to these direct grazing impacts, cattle also contribute to vegetation dynamics by the dispersal of seed. This occurs through ectozoochory (also called epizoochory), where propagules adhere to the animal's fur, or by endozoochory, where propagules pass through the animal's digestive tract and then are dispersed through feces or dung (Milotić & Hoffmann 2016). The "Foliage is the Fruit" hypothesis of Janzen (1984) suggests that characteristics such as palatability and seed production co-evolved to suit endozoochory. For some plant species, zoochory is the most successful dispersal mechanism (Malo & Suárez 1995a; Pakeman *et al.* 2002), and plants may be specialized to disperse through endozoochory (Anderson *et al.* 2014). Endozoochory by big herbivores often contributes to long-distance dispersal of plants, since many animals move large distances and can transport a large number of germinable seeds that



they then deposit in their dung (Milotić & Hoffmann 2016). Additionally, endozoochory can facilitate plant establishment as nutrients and dung organic matter may increase establishment success (Sevilla *et al.* 1996; Vignolio & Fernández 2010).

One of the main issues in grassland restoration is how to overcome seed limitation (Buisson *et al.* 2019). Many species do not have a long-term persistent soil seed bank for regeneration after their disappearance from established vegetation (Cosyns *et al.* 2005) and transient soil seed banks predominate in some communities (Funes *et al.* 2001; Márquez *et al.* 2002). Müller *et al.* (2013), in a study of semi-natural temperate grasslands, found that species diversity was more limited by the availability of propagules than by the availability of adequate sites for germination. In many restoration projects, it is therefore necessary to overcome propagule limitation of native species introduction, and different techniques for this are being used around the world (Kiehl *et al.* 2010, Palma & Laurance 2015). Cattle can contribute through endozoochory, especially if management allows for shifting the herd from well managed pastures to degraded sites (Mouisse *et al.* 2005).

Studies that quantify endozoochory are useful to assess the potential of cattle grazing as a restoration tool, but still are rare in many grassland ecosystems. For temperate grasslands, in particular in Europe, seed dispersal by endozoochory has been evaluated for different animals, including cattle, sheep, horses and rabbits (Malo & Suárez 1995a; Bakker & Olf 2003; Stroh *et al.* 2012; Mouissie *et al.* 2005). In general, these studies show that many plant species, especially graminoids, have high germination and recruitment success after dispersal by dung. Also, cattle and equine dung have greater richness and abundances of seeds compared to sheep dung. These studies also indicate seasonal variation of seed transport in dung, in consequence of the timing of reproduction of plant species. In contrast, few studies are available on seed dispersal in dung for Southern Hemisphere ecosystems (but see Sevilla *et al.* 1996 on the forage species *Lotus tenuis* and Vignolio & Fernández 2010 on exotic species, both studies

from the Argentinean Flooding Pampa). Anderson *et al.* (2014) recognized that endozoochory may be an important but neglected ecological process in tropical savannas dominated by large herbivores. Importantly, for tropical and subtropical systems, we can expect that seed transport may take place over larger parts of the year, as phenological patterns differ from those of temperate systems.

Here, we investigated seed communities in cattle dung in subtropical grassland in southern Brazil. These grasslands are rich in forb species and are characterized by the coexistence of C3 (winter) and C4 (summer) grasses (Overbeck *et al.* 2007; Andrade *et al.* 2019), groups with distinct phenological patterns and thus likely also seasonal differences in dispersal. In order to evaluate seasonal variation of potential seed transport in cattle, dung samples were taken four times over one year. The study was undertaken in paddocks with different grazing intensities, to evaluate if grazing intensity itself would influence seed transport, in consequence of differences in seed availability. We also relate the species composition of dung samples to the community composition of the studied grasslands, using data on seed production in vegetation. Overall, we expect: (a) to find larger seed dispersal in spring and summer, when the more abundant grasses are fruiting; (b) to find differences in abundance and species composition among grazing intensity treatments, both in dung and vegetation, as forage allowance percentages and stocking rates influence the grazing process and thus the plant community; and (c) to find more seeds of low-growing and more palatable graminoids and low seed abundance of woody and unpalatable species in dung samples.

## **Materials and methods**

### **Study area**

The study was conducted in an area of natural grassland located at the Agronomic Experimental Station of the Universidade Federal do Rio Grande do Sul, Eldorado do Sul, RS,

Brazil (30°06'13"S and 51°40'55"W). Climate of the region is Cfa according to the Köppen classification, i.e., with hot and humid summers, and mild to cool winters. Mean monthly precipitation during the one-year study-period was 141 mm and average temperatures ranged from 14°C to 25°C (data from Meteorological Station Experimental Agronomic Station of UFRGS; see Table 1). Poaceae, Asteraceae, Fabaceae, Cyperaceae, Rubiaceae and Apiaceae are the most important families in South Brazilian grasslands (Andrade *et al.* 2019). An important characteristic of these grasslands is the coexistence of C3 and C4 grasses which confers to the region the potential for grazing all year round, even though carrying capacity is lower in winter (Overbeck *et al.* 2007).

**Table 1.** Monthly mean precipitation and average temperatures at the study site by season. Data from Meteorological Station Experimental Agronomic Station of UFRGS.

	Temperature mean (°C)		Monthly precipitation mean (mm)
	Min.	Max.	
<b>Spring</b>	27	16	157
<b>Summer</b>	30	20	159
<b>Autumn</b>	21	11	130
<b>Winter</b>	21	10	117

In an area of 52 hectares, an experiment with different cattle stocking rates was established in 1986, i.e., it had been in place for 30 years at the time of our study. The treatments were daily forage allowances (FA) of 4, 8, 12, and 16 kg of dry matter mass (DM) per 100 kg of live weight (LW), with two replicates per FA. This means that every day 4, 8, 12 or 16% of the body weight of animals is available in forage biomass. The 4% treatment represents the highest grazing intensity (low forage allowance) and the 16% treatment represents the lowest grazing intensity (high forage allowance). The FA is calculated on the basis of total dry matter present in the pasture. The animals used in the experiment were heifers about 300 kg live weight each. The area of each experimental unit varied from 3 to 5 hectares (for details see Cruz *et al.* 2010; Fischer *et al.* 2019).

## **Dung sampling and seedling emergence**

Dung was collected every three months, in November 2015 (Southern Hemisphere spring), March 2016 (Southern Hemisphere summer), July 2016 (Southern Hemisphere autumn) and September 2016 (Southern Hemisphere winter). In each experimental unit (paddock), fresh dung was collected from five different animals, resulting in ten samples per treatment, totaling 40 samples for each season. Animals had stayed in the paddock for at least a month before the moment of sampling. Subsequently, the samples were dried at room temperature for two weeks (Cosyns *et al.* 2005).

We used the seedling emergence method for evaluation of the dung plant communities (Roberts 1981). Each dried sample was homogenized, and a total volume of 900 ml used for further analysis. The samples were placed on a 1 cm layer of sterilized substrate, in a layer of about 5 cm, and watered. Samples were kept in a greenhouse at the Faculty of Agronomy, Department of Forage Plants and Agrometeorology, UFRGS, under ambient temperature conditions. Trays with sterile soil were distributed randomly among samples to monitor possible contamination of airborne diaspores, as usually done in soil seed bank studies (Minervini-Silva & Overbeck 2020). Trays were watered when necessary. Emergent seedlings were counted weekly and removed. When seedling identification was not possible at the time of counting and removal, at least one individual of each morphospecies was transplanted into a larger pot where it was watered regularly and kept until it reached the reproductive stage and identification was possible. Scientific names of the species and families were verified through the Flora do Brasil 2020 database (<http://floradobrasil.jbrj.gov.br/>). All samples were kept in the greenhouse for an entire year.

## **Survey of vegetation**

A survey of vegetation was carried out to compare the composition of dung samples with that of seeds available in the vegetation to be able to evaluate the relationship between plant seeding and seed dispersal by cattle. Sampling was conducted in five permanent plots of 1 m<sup>2</sup> per experimental unit (paddock) and always within two weeks of the date of dung sampling. Minimum distance between plots was at least 50 m and they were situated at least 20 m from fences (adjacent treatments). In each plot, we recorded all species with mature fruits or fruits in the phase of seed liberation at the moment of sampling. Quantification of reproductive structures was sometimes difficult due to the high diversity of inflorescence types with largely divergent flower types and numbers as well as fruit and seed numbers. We counted the number of mature or dispersing reproductive structures produced from individual flowers, inflorescences (e.g. Asteraceae capitula) or inflorescence branches (e.g. Poaceae and Cyperaceae panicles). Each of the exemplified reproductive structures was considered as a unit. While this is a simplification, it does allow for an overall estimation of flowering and seed production, even though more detailed comparisons can only be done within each group of plants (e.g. within Poaceae, within Asteraceae). However, given that in many plant families not all flowers develop to fruits or that many seeds are not viable (e.g. Poaceae, Campbell 1985), a more detailed analysis also would not lead to exact data. Scientific names of the species and families were verified through the Flora do Brasil 2020 database (<http://floradobrasil.jbrj.gov.br/>).

## **Data analysis**

To characterize the community present in dung samples, all species were classified according to life cycle (perennial and non-perennial, i.e., annual and/or biannual, Burkart 1969),

principal life forms (grasses, sedges and rushes, i.e. graminoids; herbs; subshrubs/shrubs), and, in the case of graminoids, habit (caespitose and prostrate).

For all statistical analysis, the mean value of the five dung samples per paddock was used, resulting in two replicates for each grazing intensity treatment. We compared seedling number, richness, composition, life cycle and life form composition of dung samples, among seasons and four grazing intensities, by randomization tests comparing groups of sampling units, using Euclidean distance (10000 iterations). To visualize the main tendencies of variation in dung sample species among the four seasons, we performed principal coordinate analysis (PCoA) on the community data, using chord distance. The similarity of the dung sample species and vegetation composition (i.e. species with ripe seeds or in dispersal phase) for each season was evaluated using the Sørensen similarity index (Zuur *et al.* 2007). We compared the Sørensen index values, among seasons and four grazing intensities, by randomization tests comparing groups of sampling units using Euclidean distance (10000 iterations). All analyses were conducted in MULTIV (Pillar 1999).

## **Results**

### **Dung seed content**

A total of 93 taxa were recorded in the dung samples, representing 21 botanical families, with Poaceae (31% of species; 33% of total seedlings), Cyperaceae (22% of species; 46% of seedlings), Juncaceae (2% of species; 5% of seedlings) and Asteraceae (16% of species; 3% of seedlings) as the most important regarding species richness and abundance (Appendix S1). No seedling emergence was detected in any of the trays with sterile soil. *Eleocharis viridans*, *Cyperus brevifolius*, *Paspalum notatum*, *Fimbristylis dichotoma* and *Juncus tenuis* were the most important species in terms of absolute seed abundance in all seasons (Table 2).

**Table 2.** Most abundant plant species found in dung sampling, by sampling season. All species that presented more than 1.5% of total seed number (over all four sampling dates) are shown. Another 76 species showed lower total contribution to total seed number.

Family	Species	Spring	Summer	Autumn	Winter	Total	% of total seeds
Cyperaceae	<i>Eleocharis viridans</i> Kük. ex Osten	33	130	41	22	226	13.7%
Cyperaceae	<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.	54	58	83	23	218	13.2%
Poaceae	<i>Paspalum notatum</i> Flüggé	21	54	20	3	98	5.9%
Cyperaceae	<i>Fimbristylis dichotoma</i> (L.) Vahl	3	47	36	6	92	5.6%
Juncaceae	<i>Juncus tenuis</i> Willd.	17	19	27	5	68	4.1%
Cyperaceae	<i>Cyperus obtusatus</i> (J.Presl & C.Presl) Mattf. & Kük.	31	13	15	1	60	3.6%
Poaceae	<i>Paspalum pumilum</i> Nees	32	7	18	0	57	3.4%
Poaceae	<i>Axonopus affinis</i> Chase	35	17	2	1	55	3.3%
Poaceae	<i>Piptochaetium montevidense</i> (Spreng.) Parodi	26	9	5	2	42	2.5%
Poaceae	<i>Steinchisma hians</i> (Elliott) Nash	24	9	4	4	41	2.5%
Poaceae	<i>Paspalum pauciciliatum</i> (Parodi) Herter	0	11	24	4	39	2.4%
Cyperaceae	<i>Cyperus sesquiflorus</i> (Torr.) Mattf. & Kük.	31	1	1	0	33	2.0%
Asteraceae	<i>Gamochaeta coarctata</i> (Willd.) Kerguélen	1	2	1	27	31	1.9%
Iridaceae	<i>Sisyrinchium micranthum</i> Cav.	15	12	1	3	31	1.9%
Cyperaceae	<i>Bulbostylis subtilis</i> M.G.López	15	7	3	5	30	1.8%
Poaceae	<i>Digitaria violascens</i> Link	27	3	0	0	30	1.8%
Poaceae	<i>Mnesithea selloana</i> (Hack.) de Koning & Sosef	4	10	8	3	25	1.5%

The most important grass species were prostrate (*P. notatum*, *Paspalum pumilum*, *Axonopus affinis* as the three most abundant grasses). Most of the species identified in the dung samples were native species (94% of species; 96.4% of total seedlings). Of the 93 species found in dung samples, three were species considered exotic and invasive in the region (Guido *et al.* 2016): *Cynodon dactylon*, *Senecio madagascariensis* and *Eragrostis plana* (2% of species; 0.3% of total seedlings) (Appendix S1). The large majority of species (82%) that emerged from dung samples were perennial species. A total of 65 species were found in spring samples, 61 species in summer samples, 45 in autumn samples and 37 in winter samples. Proportions were similar for total seedling numbers per season, with 581 seedlings for spring, 525 seedlings for summer, 386 seedlings for autumn and 163 seedlings for winter. The randomization tests comparing groups of sampling units presented significant differences between winter and other seasons

( $p < 0.004$ ) and between spring and autumn, both considering abundance (Fig. 1a) and richness (Fig. 1b) ( $p \leq 0.007$ ; details Appendix S2). No significant differences were found for seedling abundance and species richness among the different grazing intensities ( $p > 0.05$ ; details Appendix S2).

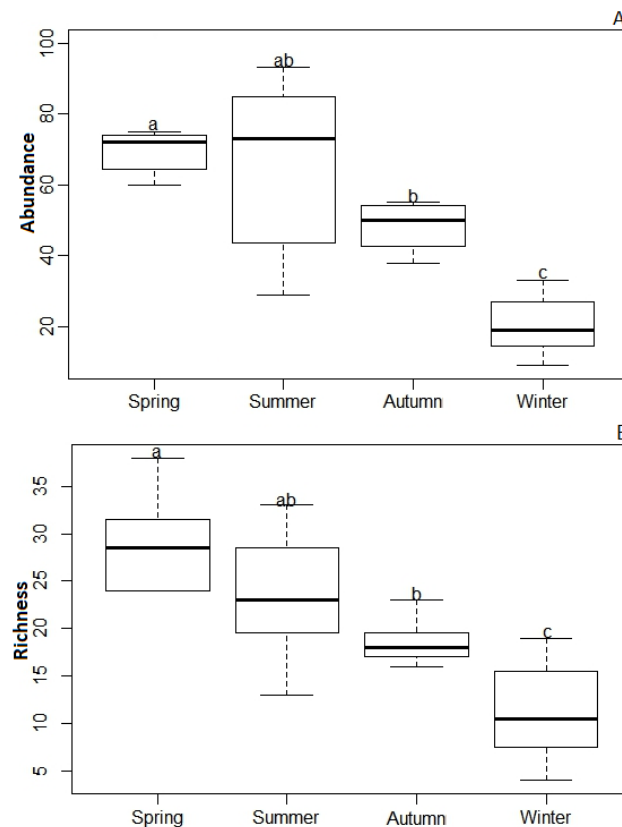
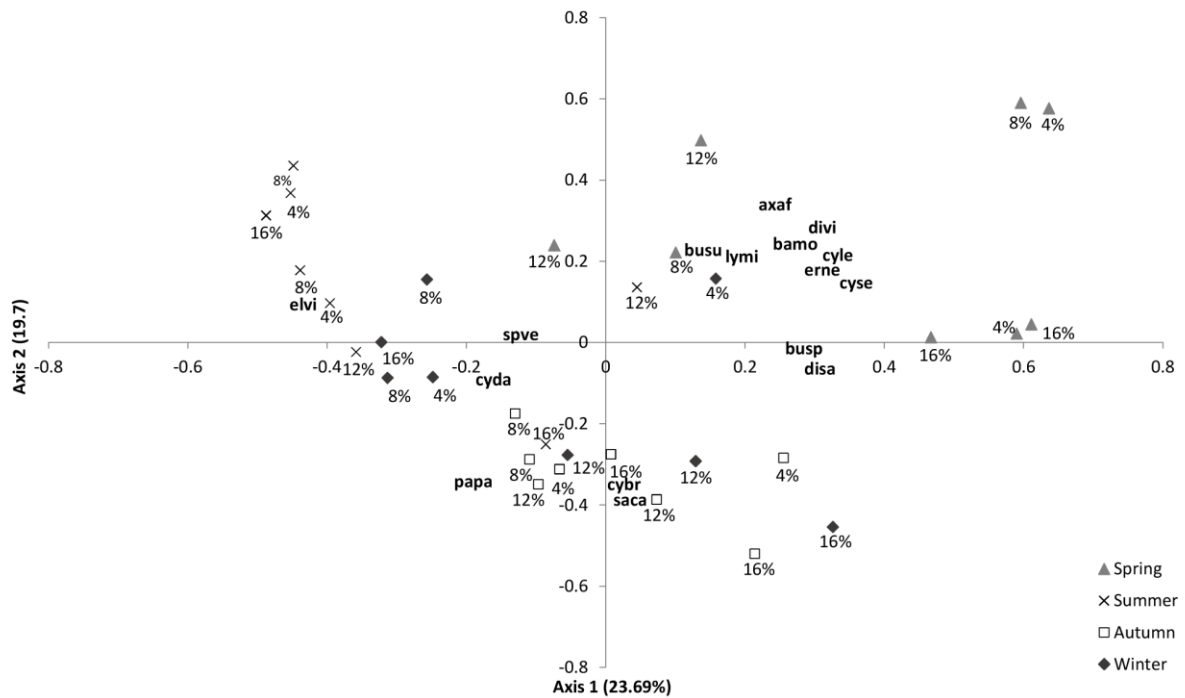


Figure 2. Boxplots of seedling abundance (a) and richness (b) found in dung samples of grazed subtropical grassland in southern Brazil, for each season. Different letters indicate significant differences among seasons.

In the principal coordinate analysis (Fig. 2), seasons separated, with especially spring distant from the other seasons. Some graminoid species had high correlation coefficients with the axes, and most were associated with the spring samples. No clear pattern regarding grazing treatments emerged. Composition differed significantly between all seasons ( $p \leq 0.029$ ; details Appendix S2), while for the grazing factor we observed significant differences between



treatment 4% (high grazing intensity) and treatments 12% ( $p= 0.0014$ ) and 16% (moderate and low grazing intensities) ( $p= 0.0013$ ; details Appendix S2).



**Figure 2.** Ordination diagram (PCoA) of dung samples composition of grazed subtropical grassland in southern Brazil, by season (symbols). Percentage values next to symbols, refer to forage allowance (see text for detail). Four letter codes represent the abbreviated names of the species with highest ( $> 0.535$ ) correlation coefficients (axaf: *Axonopus affinis*; bamo: *Bacopa monnieri*; busp: *Bulbostylis sphaerocephala*; busu: *Bulbostylis subtilis*; cybr: *Cyperus brevifolius*; cyda: *Cynodon dactylon*; cyle: *Cyclosporum leptophyllum*; cyse: *Cyperus sesquiflorus*; disa: *Dichanthelium sabulorum*; divi: *Digitaria violascens*; elvi: *Eleocharis viridans*; erne: *Eragrostis neesii*; ly mi: *Lysimachia minima*; papa: *Paspalum pauciciliatum*; saca: *Sacciolepis campestris*; spve: *Spermacoce verticillata*).

Perennials dominated in all seasons, contributing, with the exception of the spring samples in the 4% treatment, with more than 70% of seedlings (Fig. 3). Both perennials and non-perennials showed variation in seedlings abundances among winter and the other seasons (Appendix S2). In the treatment with the highest grazing intensity (4% FA), non-perennials were more important in spring and winter and, to a lesser extent, in autumn (Fig. 3). However, considering the grazing factor, we did not find significant differences for non-perennials

(Appendix S2), while the perennials showed significant differences between treatment 4% (high intensity) and treatment 12% (moderate intensity) (Appendix S2).

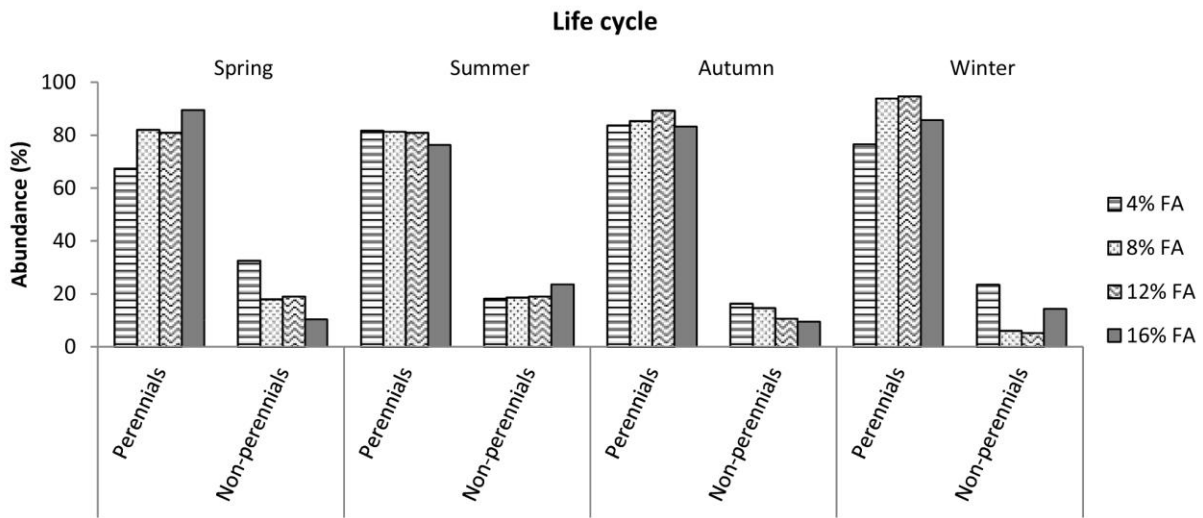


Figure 3. Percentage of seedling abundance according to life cycle in dung samples from grazed subtropical grassland in southern Brazil, by season and forage allowance (FA) treatment.

In all seasons, grasses and the group of sedges and rushes were the most abundant life form groups in terms of seedling production, with sedges and rushes contributing more in all seasons than any other group except in spring (Fig. 4; Appendix S2). Herbs were more important in spring and winter, while shrubs showed low abundances throughout the year (Fig 4; Appendix S2). Grasses, sedges and rushes and herbs varied mainly between the high grazing intensity (4% FA) and the moderate and low grazing intensities (12% and 16% FA) (Appendix S2).

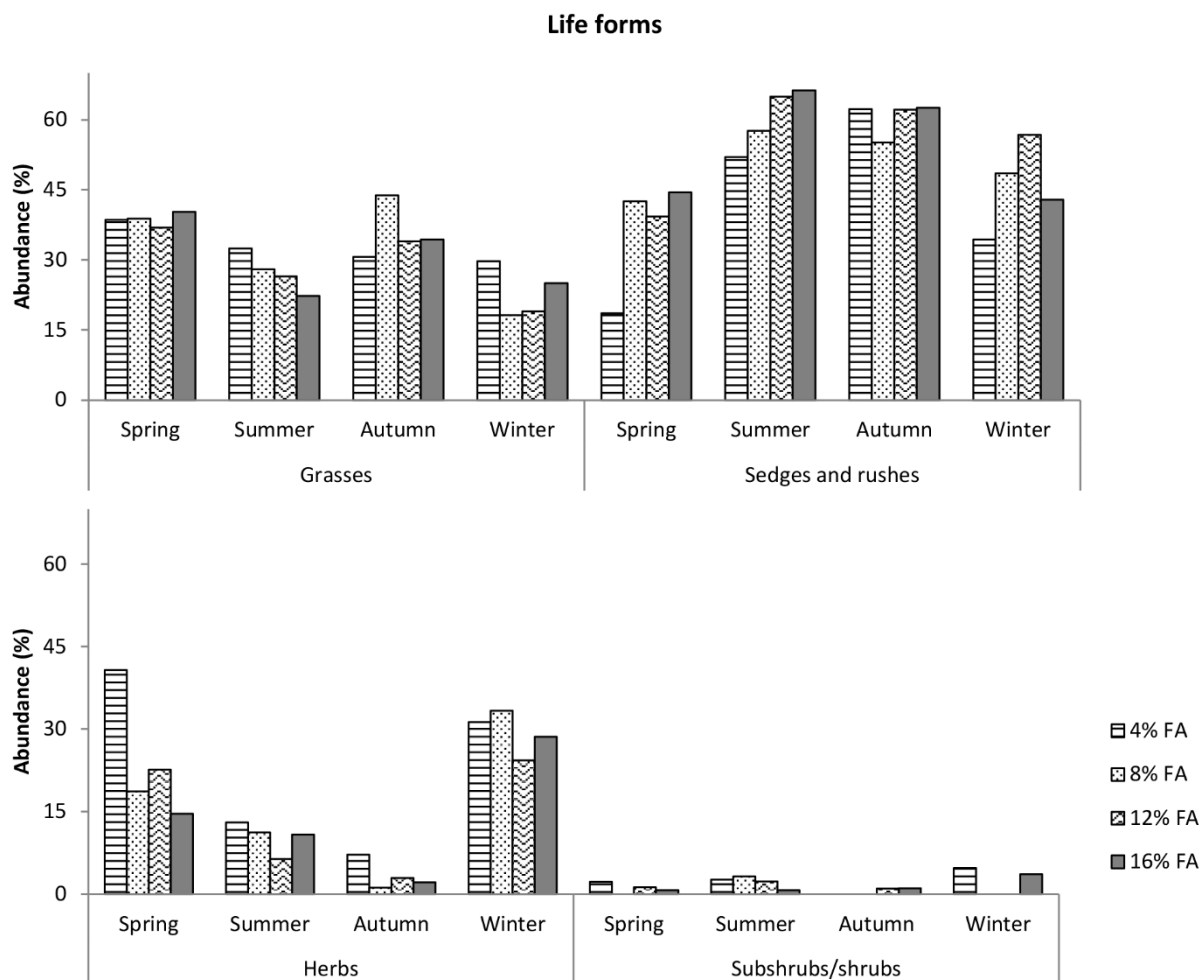


Figure 4. Percentage of seedling abundance according to life form in dung samples from grazed subtropical grassland in southern Brazil, by season and forage allowance (FA) treatment.

### Relation between dung seed bank and vegetation

In the vegetation survey, conducted in the same months as the dung samplings, 90 species were found, across the four treatments, dispersing seed or fruiting (Appendix S3). The vegetation survey species were distributed in 20 families, with highest contribution of species from the Poaceae (59%), Asteraceae (14%) and Cyperaceae (8%). *Steinchisma hians*, *Setaria parviflora*, *Andropogon lateralis*, *Axonopus affinis*, *Paspalum notatum*, *Mnesithea selleana* and *Baccharis crispa* were the species with highest levels of seed dispersal or mature fruits (Appendix S3). While maturity/dispersal clearly peaked in spring and autumn, the values found

were relatively similar among seasons, except for winter when number of species with mature fruits and in dispersion was lowest. Of the 90 species identified in the vegetation surveys, 43 species were also found in the dung samples. An additional 50 species were found in the dung samples, indicating that our vegetation sampling was not sufficient, in terms of sampling effort, to capture all plant species available to cattle to graze. In consequence, floristic similarities between dung samples and established vegetation treatments were rather low, and especially in winter (Table 3).

**Table 3.** Results of Sørensen similarity index among dung samples and the seed-producing vegetation component (i.e. species with ripe seeds or in seed dispersal) for each forage allowance treatment and season (A). Results of randomization tests (MULTIV, Pillar 1999) comparing Sørensen similarity index values among seasons and forage allowance treatments (B). The symbol \* indicates significant differences ( $p \leq 0.05$ ) among seasons and forage allowance treatments (FA).

<b>A.</b>			<b>Treatments (% FA)</b>			
<b>Seasons</b>	<b>4%</b>	<b>8%</b>	<b>12%</b>	<b>16%</b>		
<b>Spring</b>	28%	26%	15%	29%		
<b>Summer</b>	29%	31%	30%	23%		
<b>Autumn</b>	24%	25%	22%	19%		
<b>Winter</b>	7%	0%	0%	0%		

<b>B.</b>					
<b>Source of variation</b>	<b>Sum of squares (Q)</b>	<b>P-values *</b>	<b>Source of variation</b>	<b>Sum of squares (Q)</b>	<b>P-values *</b>
<i>Between groups</i>	0.013968	0.355	<i>Between groups</i>	0.33991	0.001 *
4% - 8%	0.00068632	0.707	Spring - Summer	0.0055011	0.377
4% - 12%	0.011085	0.123	Spring - Autumn	0.0017434	0.636
4% - 16%	0.0063826	0.195	Spring - Winter	0.20745	0.001 *
8% - 12%	0.006255	0.325	Summer - Autumn	0.013438	0.116
8% - 16%	0.002883	0.434	Summer - Winter	0.28052	0.004 *
12% - 16%	0.00064491	0.73	Autumn - Winter	0.17116	0.001 *

## Discussion

### Seasonal patterns of seed dispersal by cattle dung

Our study is the first to investigate seed transport in cattle dung in subtropical grasslands, species-rich ecosystems with considerable seasonal variation in seed production. In

the dung samples, we found viable seeds from a large number of plant species that represent the typical botanical families of the subtropical grassland system here studied. These results indicate that cattle endozoochory can contribute to grassland vegetation dynamics.

Seed abundance in dung was similar for spring and summer but dropped in autumn and winter, as we had expected. The spring and summer densities were similar to what has been found in other studies of seed in dung in temperate grassland (Mouissie *et al.* 2005) and in the Argentinean Flooding Pampa (Vignolio & Fernández 2010). While we did observe clear seasonality, the subtropical grasslands studied by us show a large number of seeds in dispersal via dung in spring, summer and autumn, and some dispersal even occurring in winter when richness and abundance values were the lowest. The winter seed community in dung was dominated by ruderal herbs such as *Chaptalia sp.*, *Chevreulia sarmentosa*, *Elephantopus mollis* and *Hypoxis decumbens* as well as Cyperaceae; these species are especially abundant in the 4% FA treatment, which can be considered as overgrazed (Fedrigo *et al.* 2018). In short, seed density and species richness in dung samples as well as composition varied significantly over the year.

### **Variation of functional characteristics of species transported by cattle dung in response to seasons and distinct grazing intensities**

Our results clearly show variation of the dung seed community across seasons and, to a lesser extent, across grazing treatments: the high grazing intensity treatment (4% FA) was the most distinct treatment. The general finding of higher abundances of perennial species in comparison to non-perennial species was expected given that the large majority of the grasslands species found in our system are perennials. According to Vignolio & Fernández (2010), endozoochory seems to favor the propagation of some species of low-quality forage and ruderal plants, and our results support this: extreme conditions of grazing (very high or very

low intensity) increase the presence of annual species. Still, overall number of annual species was low, which – apart from the fact that annuals are rather rare in our study system (Overbeck & Pfadenhauer 2007) may be due to the fact that generally they are ruderal with low stature, which may impede ingestion of seeds by cattle.

Similar to our study, several previous studies have observed a greater contribution of graminoids in endozoochory in grasslands systems (Malo and Suárez 1995b; Vignolio & Fernández 2010; Anderson *et al.* 2014; Milotić & Hoffmann 2016). In winter and spring, when forage available is lower, herbs had somewhat greater importance. Also, the lower and more open communities with lower competition due to the more intense grazing by cattle in treatments with high (4% FA) and moderate (8%) grazing intensities may have contributed to the higher percentage of herbs in spring and winter. The main reason for this pattern likely is selective grazing by cattle that prefer low-growing and more palatable graminoids (Malo & Suárez 1995b; Nabinger *et al.* 2009). In addition, Milotić & Hoffmann (2016) verified that seeds of Cyperaceae and Poaceae were hardly harmed by treatments simulating passage through a digestive system, which therefore may lead to higher survival of species from this group when compared to herbs. Gardener *et al.* (1993), working with a large number of tropical legumes and grasses, showed that the germination of *Digitaria ciliaris*, *Axonopus affinis* and *Paspalum notatum* – prostrate species also present in our grassland– increased after passage through the digestive tract, in contrast to caespitose grasses that did not survive passage through cattle. Possibly, these former species do not form a large seed bank precisely due to the fact that they are abundant, well adapted even to heavy grazing and long-lived, i.e., mortality should be low (Medeiros 2000), and, as indicated by our study, disperse well. Indeed, *P. notatum* was much more frequent in the dung samples than in a study of the soil seed bank at the study site (Minervini-Silva & Overbeck 2020). Importantly, the most abundant grasses in the dung samples were prostrate grasses (e.g. *P. notatum*, *P. pumilum*, *P. pauciciliatum* and *A. affinis*).

From the group of caespitose grasses, the low-stature and ruderal grass *Piptochaetium montevidense* was the most important. It appears that chances of transport of the tall growing tussock grasses, e.g. *Andropogon lateralis*, to just cite the most dominant species in our system, are low, even though they also are an important component of the grasslands studied by us. This can be related to both the grazing behavior of cattle that preferably forage on the lower stratum of these grasslands, and by the fact that these species present a more conservative growth strategy (Cruz *et al.* 2010). Given the low contribution of these species in the soil seed bank in the region (Vieira *et al.* 2015; Minervini-Silva & Overbeck 2020), and their apparent lack of dispersal, as found here, we can conclude that these species do not depend much on new recruitment for maintenance of their populations. However, this will also mean that they do not easily colonize sites where they are absent. For rushes and sedges, also an important species group in vegetation, we found that seed transport is disproportionately higher, just as species from this group are found in large extent in the soil seed bank (Vieira *et al.* 2015; Minervini-Silva & Overbeck 2020). It thus becomes clear – as would be expected – that dung transport favors species with a ruderal character, i.e. high dispersal capacity.

The dung samples included exotic and native species. In the study by Vignolio & Fernández (2010), 32% of the species found were exotic. In our case, the number was lower, but of course comparisons among studies are difficult as the relation between native and exotic species depends on the contribution of species from both groups in vegetation and in seed production. Additionally, presence of exotic species at a given site depends on many factors beyond the site scale, such as regional land use patterns or land use history (Guido *et al.* 2016). In our study, cattle did not forage at spots with a high proportion of exotic species, as patches with high cover of exotics were absent in the experimental paddocks. Nonetheless, some of these species were dispersed in cattle dung and underlines that endozoochory can also be an invasion pathway (Vavra *et al.* 2007).

### **Relationship of the dung samples to availability of seed in the grassland community**

The values of compositional similarity between dung seed communities and species found to be in dispersal in the established vegetation found by us certainly are influenced by sampling effort of both communities. Low values may be indicative that sampling effort was low, even though selectivity of cattle grazing also should be of relevance. Species that have seeds with special dispersal adaptations, such as a pappus in Asteraceae – including many shrub species that cattle does not graze on – and awns in Poaceae, were less frequent in the communities that germinated from dung than species without any obvious adaptation to wind dispersal (see also Malo & Suárez 1995a). Possibly, seeds from these wind-adapted species that are present in the vegetation may lose their viability after going through the digestive tract of the animal (Gardener *et al.* 1993). According to Janzen's theory (1984), cattle consume the seeds that are mixed with the foliage. Consequently, the species with greater abundance in the vegetation should be distributed more frequently, as also shown by Bakker & Olff (2003) and Auffret *et al.* (2012) – in winter, dispersal rates are low, and this was the period with very low similarity between the seed dung community and the component of plants in dispersal. Other studies have also found higher similarity in the season with highest abundance of plants dispersing (Malo & Suárez 1995a). However, in the case of their study, this result may also reflect that they evaluated dispersal by different grazing animals (cattle, two deer species, rabbits) with different food preferences (Malo & Suárez 1995a). Also, they worked in a system with a much more concentrated phenological pattern in their more seasonal system which should lead to a greater similarity of plants emerged from dung samples and plants in the dispersal phase in the vegetation. It seems important to conduct further studies on the specific potential of seed transport of species with high importance in the plant community and for grassland productivity, such as important forage species (e.g. Sevilla *et al.* 1996); this could even be done experimentally.



## **Can seed transport in cattle dung contribute to grassland restoration?**

Our results indicate that cattle can help overcome seed limitation in restoration projects due to seed dispersal by dung. Importantly, in subtropical grassland as studied by us, this contribution of cattle is not limited to one season, but occurs throughout the year, with clearly a smaller contribution only in winter when plant development is slow and few plants reproduce. This role of cattle thus adds to other positive effects it may have in grassland restoration projects, such as providing organic matter, control of plant biomass through consumption and trampling and opening of space in the vegetation (e.g. Silveira *et al.* 2020). Dispersal was maximized in spring and summer, considering both the richness and the abundance of species and life forms in vegetation. In contrast, grazing treatments influenced which species were transported, but not number of species and seeds. Across treatments, we verified that most seedlings that emerged were graminoids. This is important in terms of restoration, because especially grasses form the vegetation matrix of grasslands and thus can be considered target species for restoration. Clearly, more ruderal species, including sedges and rushes, made up a large part of the dung seed community as well. In our study, percentage of exotic species was low, obviously in consequence of the low percentage of exotics at our study site. Given the wide distribution of exotics, often invasive species, however, caution will need to be taken when using cattle as a restoration tool if it also has access to areas with the presence of invasive species. On the other hand, letting cattle graze in areas with availability of seeds from target species, e.g. after temporary grazing deferment that should increase seed production, before taking them to degraded areas may be used to increase seed input; knowledge on seasonality of seed production of target species would be helpful here.

Due to the overall rather low similarity between the species composition of the vegetation and the dung samples, it might be important, in restoration, to introduce some species by other means as well, especially those of underrepresented groups such as herbs or of tall-

growing grasses that apparently are not transported by endozoochory. Despite these limitations, we can conclude that, as long as seeds of native grassland species are not commercially available (Overbeck *et al.* 2013) or as long as no other efficient techniques of seed introduction exist (e.g. hay transport, Thomas *et al.* 2019a, 2019b), cattle may be used as a rather efficient restoration agent at least for reestablishment of part of the grassland plant community.

## References

- Adler, P. B., Raff, D. A. & Lauenroth, W. K. (2001). The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia* **128**(4), 465-479.
- Anderson, T. M., Schütz, M. & Risch, A. C. (2014). Endozoochorous seed dispersal and germination strategies of Serengeti plants. *J. Veg. Sci.* **25**, 636-647.
- Andrade, B. O., Bonilha, C. L., Overbeck, G. E., Vélez-Martin, E., Rolim, R. G., Bordignon, S. A. L., Schneider, A. A., Vogel Ely, C., Lucas, D. B., Garcia, E. N., Santos, E. D., Torchelsen, F. P., Vieira, M. S., Silva Filho, P. J. S., Ferreira, P. M. A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V. D. & Boldrini, I. I. (2019). Classification of South Brazilian grasslands: Implications for conservation. *Appl. Veg. Sci.* **22**, 168-184.
- Auffret, A. G., Schmucki, R., Reimark, J. & Cousins, S. A. O. (2012). Grazing networks provide useful functional connectivity for plants in fragmented systems. *J. Veg. Sci.* **23**, 970-977.
- Bakker, E. S. & Olff, H. (2003). Impact of different-sized herbivores on recruitment opportunities for subordinate herbs in grasslands. *J. Veg. Sci.* **14**, 465-474.
- Campbell, C. S. (1985). The Subfamilies and Tribes of Gramineae (Poaceae) in the Southeastern United States. *J. Arnold Arbor.* **66**(2), 123-199.
- Buisson, E., Le Stradic, S., Silveira, F. A. O., Durigan, G., Overbeck, G. E., Fidelis, A., Fernandes, G. W., Bond, W. J., Hermann, J. M., Mahy, G., Alvaredo, S. T., Zaloumis, N. P. &

Veldman, J. W. (2019). Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. *Biol. Rev.* **94(2)**, 590-609.

Burkart, A. (1969). *Flora ilustrada de Entre Rios (Argentina)*. Buenos Aires: Instituto Nacional de Tecnología Agropecuaria.

Cosyns, E., Claerbout, S., Lamoot, I. & Hoffmann, M. (2005). Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecol.* **178(2)**, 149-162.

Cruz, P., Quadros, F. L. F., Theau, J. P., Frizzo, A., Jouany, C., Duru, M. & Carvalho, P. C. F. (2010). Leaf traits as functional descriptors of the intensity of continuous grazing in native grasslands in the south of Brazil. *Rangeland Ecol. Manage.* **63**, 350-358.

Da Trindade, J. K., Pinto, C. E., Neves, F. P., Mezzalira, J. C., Bremm, C., Genro, T. C. M, Tischler, M. R., Nabinger, C., Gonda, H. L. & Carvalho, P. C. (2012). Forage allowance as a target of grazing management: implications on grazing time and forage searching. *Rangeland Ecol. Manage.* **65(4)**, 382-393.

Fedrigo, J. K., Ataíde, P. F., Azambuja-Filho, J., Oliveira, L. V., Jaurena, M., Laca, E. A., Overbeck, G. E. & Nabinger, C. (2018). Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. *Restor. Ecol.* **26(4)**, 677-685.

Fischer F. M., Bonnet O. J. F., Cezimbra, I. M. & Pillar, V. D. (2019). Long-term effects of grazing intensity on strategies and spatial components of functional diversity in subtropical grassland. *Appl. Veg. Sci.* **22**, 39-47.

Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro. <http://floradobrasil.jbrj.gov.br/>.

Funes, G., Basconcelo S., Díaz S. & Cabido, M. (2001). Edaphic patchiness influences grassland regeneration from the soil seed-bank in a mountain grasslands of central Argentina. *Austral Ecol.* **26**, 205-212.

- Gardener, C. J., McIvor, J. G. & Jansen, A. (1993). Survival of seeds of tropical grassland species subjected to bovine digestion. *J. Appl. Ecol.* **30(1)**, 75-85.
- Gasparino, D., Malavasi, U. C., Malavasi, M. M. & Souza, I. (2006). Quantificação do banco de sementes sob diferentes usos do solo em área de domínio ciliar. *Rev. Árvore* **30(1)**, 1-9.
- Guido, A., Vélez-Martin, E., Overbeck, G. E. & Pillar, V. D. (2016). Landscape structure and climate affect plant invasion in subtropical grasslands. *Appl. Veg. Sci.* **19(4)**, 600-610.
- Janzen, D. H. (1984). Dispersal of small seeds by big herbivores: foliage is the fruit. *Am. Nat.* **123**, 338-353.
- Kiehl, K., Kirmer, A., Donath, T. W., Rasran, L. & Hölzel, N. (2010). Species introduction in restoration projects - evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic Appl. Ecol.* **11**, 285-299.
- Kinucan, R. J. & Smeins, F. E. (1992). Soil seed bank of a semiarid Texas grassland under three long-term (36 years) grazing regimes. *Am. Midl. Nat.* **128**, 11-21.
- Loydi, A. (2019). Effects of grazing exclusion on vegetation and seed bank composition in a mesic mountain grassland in Argentina. *Plant Ecol. Divers.* **12 (2)**, 127-138.
- Malo, J. E. & Suárez, F. (1995a). Herbivorous mammals as seed dispersers in a Mediterranean *dehesa*. *Oecologia* **104**, 246-255.
- Malo, J. E. & Suárez, F. (1995b). Establishment of pasture species on cattle dung: the role of endozoochorous seeds. *J. Veg. Sci.* **6**, 169-174.
- Márquez, S., Funes, G., Cabido, M. & Pucheta, E. (2002). Efectos del pastoreo sobre el banco de semillas germinable y la vegetación establecida en pastizales de montaña del centro Argentina. *Rev. Chil. Hist. Nat.* **75**, 327-337.
- Medeiros, R. B. (2000). Bancos de sementes no solo e dinâmica vegetal. In: *XVIII Reunião do Grupo Técnico em Forrageiras do Cone Sul – Zona Campos Guarapuava*, 61-87.

- Milotić, T. & Hoffmann, M. (2016). How does gut passage impact endozoochorous seed dispersal success? Evidence from a gut environment simulation experiment. *Basic Appl. Ecol.* **17**, 165-176.
- Minervini-Silva, G. H. & Overbeck, G. E. (2020). Soil seed bank in a subtropical grassland under different grazing intensities. *Acta Bot. Bras.* **34(2)**, 360-370.
- Mouissie, A. M., Vos, P., Verhagen, H. M. C. & Bakker, J. P. (2005). Endozoochory by free-ranging, large herbivores: ecological correlates and perspectives for restoration. *Basic Appl. Ecol.* **6**, 547-558.
- Müller, J., Heinze, J., Joshi, J., Boch, S., Klaus, V. H., Fischer, M. & Prati, D. (2013). Influence of experimental soil disturbances on the diversity of plants in agricultural grasslands. *J. Plant Ecol.* **7(6)**, 506-517.
- Nabinger, C., Ferreira, E. T., Freitas, A. K., Carvalho, P. C. F. & Sant'Anna, D. M. (2009). Produção animal com base no campo nativo: aplicações de resultados de pesquisa In: Pillar, V. D., Müller, S. C., Castilhos, Z. & Jacques, A. V. A. (eds.) *Campos Sulinos: conservação e uso sustentável da biodiversidade*. Brasília: Ministério do Meio Ambiente, pp. 78-87.
- Overbeck, G. E., Müller, S. C., Fidelis, A., Pfadenhauer, J., Pillar, V. D., Blanco, C., Boldrini, I. I., Both, R. & Forneck, E. (2007). Brazil's neglected biome: The South Brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* **9**, 101–116.
- Overbeck, G. E., Hermann, J. M., Andrade, B. O., Boldrini, I. I., Kiehl, K., Kirmer, A., Koch, C., Kollmann, J., Meyer, S. T., Müller, S. C., Nabinger, C., Pilger, G. E., Trindade, J. P. P., Vélez-Martin, E., Walker, E. A., Zimmermann D. G. & Pillar, V. D. (2013). Restoration ecology in Brazil-time to step out of the forest. *Nat. Conserv.* **11**, 92-95.
- Overbeck, G. E. & Pfadenhauer, J. (2007). Adaptive strategies in burned subtropical grassland in southern Brazil. *Flora* **202**, 27-49.

- Pakeman, R. J., Digneffe, G. & Small, J. L. (2002). Ecological correlates of endozoochory by herbivores. *Funct. Ecol.* **16**, 296-304.
- Palma A.C. & Laurance S.G.W. (2015). A review of the use of direct seeding and seedling plantings in restoration: what do we know and where should we go? *Appl. Veg. Sci.* **18**, 561-568.
- Pillar, V. D. (1999). MULTIV, software for multivariate analysis and randomization testing. Porto Alegre: Departamento de Ecologia, UFRGS. <http://143.54.23.101/Software/Multiv>.
- Roberts, H. A. (1981). Seed banks in the soil. *Advances in Applied Biology*. Cambridge: Academic Press. **6**, pp. 55.
- Sevilla, G. H., Fernández, O. N., Minõn, D. P. & Montes, L. (1996). Emergence and seedling survival of *Lotus tenuis* in *Festuca arundinacea* pastures. *J. Range Manage.* **49**, 509-511.
- Silveira, F. A. O., Arruda, A. J., Bond, W., Durigan, G., Fidelis, A., Kirkman, K., Oliveira, R. S., Overbeck, G. E., Sansevero, J. B. B., Siebert, F., Siebert, S. J., Young, T. P., Buisson, E. (2020), Myth-busting tropical grassy biome restoration. *Restor. Ecol.* **28**, 1067-1073.
- Stroh, P. A., Mountford, J. O. & Hughes, F. M. R. (2012). The potential for endozoochorous dispersal of temperate fen plant species by free-roaming horses. *Appl. Veg. Sci.* **15**, 359-368.
- Thomas, P. A., Schüller, J., Boavista, L. da R., Torchelsen, F. P., Overbeck, G. E. & Müller, S. C. (2019). Controlling the invader *Urochloa decumbens*: subsidies for ecological restoration in subtropical Campos grassland. *Appl. Veg. Sci.* **22(1)**, 96-104.
- Thomas, P. A., Overbeck, G. E. & Müller, S. C. (2019). Restoration of abandoned subtropical highland grasslands in Brazil: mowing produces fast effects, but hay transfer does not. *Acta Bot. Bras.* **33(3)**, 405-411.
- Vieira, M. S., Bonilha, C. L., Boldrini, I. I. & Overbeck, G. E. (2015). The seed bank of subtropical grasslands with contrasting land-use history in southern Brazil. *Acta Bot. Bras.* **29(4)**, 543-552.

Vavra, M., Parks, C. G. & Wisdom, M. J. (2007). Biodiversity, exotic plant species, and herbivory: The good, the bad, and the ungulate. *For. Ecol. Manage.* **246**, 66–72

Vignolio, O. R. & Fernández, O. N. (2010). Cattle dung as vector of spreading seeds of exotic species in the Flooding Pampa grasslands (Buenos Aires, Argentina). *Ann. Bot. Fenn.* **47**, 14-22.

Zuur, A., Ieno, E. N. & Smith, G. M. (2007). *Analysing Ecological Data*. New York Inc: Springer-Verlag.

## Appendices

**Appendix S1.** Abundance of seedlings (absolute number) in dung samples from grazed subtropical grassland in southern Brazil, by season and forage allowance treatment (FA; see text for detail). <sup>1</sup>Invasive species; <sup>°</sup>Exotic species.

Family	Species	Spring				Summer				Autumn				Winter			
		4%	8%	12%	16%	4%	8%	12%	16%	4%	8%	12%	16%	4%	8%	12%	16%
Amaryllidaceae	<i>Nothoscordum montevidense</i> Beauverd		2	5	1	1											
Apiaceae	<i>Centella asiatica</i> (L.) Urb.	8	3	2		3				2				3			
	<i>Cyclospermum leptophyllum</i> (Pers.) Sprague	4	1	2													
Araliaceae	<i>Hydrocotyle exigua</i> Malme		1				1										
	Asteraceae sp. 1				1												
	Asteraceae sp. 2	1															
	<i>Chaptalia piloselloides</i> (Vahl) Baker													1			
	<i>Chromolaena squarrosa</i> (Hook. & Arn.) R.M.King & H.Rob.	1															
	<i>Erechtites valerianifolius</i> (Link ex Spreng.) DC.			1													
	<i>Gamochaeta americana</i> (Will.) Wedd.			1	1												
Asteraceae	<i>Gamochaeta coarctata</i> (Willd.) Kerguelen			1			1	1		1				7	9	8	3
	<i>Gamochaeta pensylvanica</i> (Willd.) Cabrera													1			
	<i>Micropsis spathulata</i> (Pers.) Cabrera					1											
	<i>Senecio brasiliensis</i> (Spreng.) Less.													3			1
	<i>Senecio madagascariensis</i> Poir. <sup>1</sup>	2		1													
	<i>Soliva sessilis</i> Ruiz & Pav.	3	1	2	1									1			
	<i>Stenachaenium campestre</i> Baker			1													
	<i>Vernonanthura nudiflora</i> (Less.) H.Rob.	1															
Brassicaceae	<i>Cardamine chenopodiifolia</i> Pers.									2		1					1
Caryophyllaceae	<i>Stellaria media</i> (L.) Vill.	3	1		2												
Convolvulaceae	<i>Dichondra sericea</i> Sw.		1	1													
	<i>Bulbostylis sphaerocephala</i> (Boeckeler) Lindm.	2	1	3				1		3							
	<i>Bulbostylis subtilis</i> M.G.López	4	8	2	1	3	1	2	1		1	1	1	4	1		
	<i>Carex bonariensis</i> Desf. ex Poir.						1										
Cyperaceae	<i>Carex phalaroides</i> Kunth	1	1	4	1			1		3			1				
	<i>Carex sororia</i> Kunth						1										
	Cyperaceae sp. 1						1	3	1								



	<i>Cyperus aggregatus</i> (Willd.) Endl.									1		2					
	<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.	3	14	18	19	2	7	20	29	14	19	25	25	3	5	7	8
	<i>Cyperus iria</i> L.								1								
	<i>Cyperus lanceolatus</i> Poir.			1					2			1	4				1
	<i>Cyperus luzulae</i> (L.) Retz.						2	2	3			1	2				
	<i>Cyperus sesquiflorus</i> (Torr.) Mattf. & Kük.	8	6	5	12				1	1							
	<i>Cyperus obtusatus</i> (J.Presl & C.Presl) Mattf. & Kük.	3	11	8	9	1	6	5	1	6	4	3	2		1		
	<i>Eleocharis sellowiana</i> Kunth			2					1								
	<i>Eleocharis viridans</i> Kük. ex Osten	2	8	8	15	25	30	46	29	9	9	15	8	6	5	9	2
	<i>Fimbristylis autumnalis</i> (L.) Roem. & Schult.	1			1		2	4	1	1	3	2					
	<i>Fimbristylis dichotoma</i> (L.) Vahl			3		5	9	16	17	10	8	7	11	4	1	1	
	<i>Rhynchospora brittonii</i> Gale		3		1		4	7	3	1		4					
	<i>Rhynchospora tenuis</i> Link						1	1				1					
	<i>Scleria distans</i> Poir.						1		2							2	
	<i>Euphorbia selloi</i> (Klotzsch & Garcke) Boiss.					3	2	2	3			1					
Euphorbiaceae	<i>Tragia bahiensis</i> Müll.Arg.	2	1											1			
	<i>Desmodium incanum</i> DC.						2		1								
Fabaceae	<i>Stylosanthes leiocarpa</i> Vogel					1		1									
Hypoxidaceae	<i>Hypoxis decumbens</i> L.	1	3	5	1		1							1			
	<i>Sisyrinchium micranthum</i> Cav.	5	2	4	4	1	1	4	6				1	2			1
Iridaceae	<i>Sisyrinchium ostenianum</i> Beauverd	1									1						
	<i>Juncus microcephalus</i> Kunth		1	5				1	2			1	1		2		
Juncaceae	<i>Juncus tenuis</i> Willd.	1	4	7	5	4	6	5	4	12	5	6	4	1	1	2	1
Oxalidaceae	<i>Oxalis conorrhiza</i> Jacq.	2	3	5	9							1					2
	<i>Bacopa monnieri</i> (L.) Wettst.	7	1														
Plantaginaceae	<i>Mecardonia procumbens</i> var. <i>tenella</i> (Cham. & Schltdl.) V.C.Souza				1				1							1	
	<i>Plantago myosuroides</i> Lam.	10	2	3		1											
	<i>Plantago tomentosa</i> Lam.						1										
	<i>Andropogon lateralis</i> Nees							1		1	2	4	3				
	<i>Axonopus affinis</i> Chase	10	10	7	8	4	7	5	1	1	1			1			
	<i>Chascolytrum poomorphum</i> (J. Presl) L. Essi, Longhi-Wagner & Souza-Chies			1	1												
Poaceae	<i>Chascolytrum subaristatum</i> (Lam.) Desv.							1									
	<i>Cynodon dactylon</i> (L.) Pers. °						1		1				1				
	<i>Dichanthelium sabulorum</i> (Lam.) Gould & C.A. Clark	1	5	3	2					1	2	2	1				

	<i>Digitaria ciliaris</i> (Retz.) Koeler	1		3					1				1				
	<i>Digitaria violascens</i> Link	6	8	8	5	1	2										
	<i>Eleusine tristachya</i> (Lam.) Lam.			1		1							1	1	1		
	<i>Eragrostis lugens</i> Nees				1					1			1				
	<i>Eragrostis neesii</i> Trin.	5	2	1				1		1							
	<i>Eragrostis plana</i> Nees <sup>1</sup>												1				
	<i>Mnesithea selloana</i> (Hack.) de Koning & Sosef	1	1		2	1	1	5	3	3	3	1	1		1	1	1
	<i>Nassella neesiana</i> (Trin. & Rupr.) Barkworth				1			1		4	3	1		3	1	1	
	<i>Paspalum leptum</i> Schult.							1									
	<i>Paspalum notatum</i> Flüggé	2	2	4	13	10	15	17	12	4	4	9	3	1			2
	<i>Paspalum pauciciliatum</i> (Parodi) Herter					2	3	2	4	3	7	6	8	1	2		1
	<i>Paspalum paucifolium</i> Swallen	1						1									
	<i>Paspalum plicatulum</i> Michx.	1					1	2	1		1						
	<i>Paspalum pumilum</i> Nees	7	11	7	7	3	1	2	1	4	6	5	3				
	<i>Piptochaetium montevidense</i> (Spreng.) Parodi	8	6	6	6	2	1	4	2		1	2	2	1		1	
	<i>Poa annua</i> L. °	3	1	7	2									5			
	Poaceae sp. 1	1			1												
	<i>Sacciolepis vilvoidea</i> (Trin.) Chase				1					3	2	4	7				
	<i>Setaria parviflora</i> (Poir.) M.Kerguelen			3	3		1	1	2	2	2	1	2	2		3	2
	<i>Setaria vaginata</i> Spreng.					1							1				
	<i>Sporobolus indicus</i> (L.) R.Br.	1	1	1						2	1						
	<i>Steinchisma hians</i> (Elliott) Nash	4	5	9	6		2	2	5	1	3			2	1		1
Polygonaceae	<i>Polygonum punctatum</i> Elliott							1					1	1			
Primulaceae	<i>Lysimachia minima</i> (L.) U. Manns & Anderb.	5	1	1			2		3								
	<i>Borreria verticillata</i> (L.) G.Mey.						1							3			
	<i>Galianthe fastigiata</i> Griseb.													1			
Rubiaceae	<i>Galium humile</i> Cham. & Schltl.						1	1				1	1				
	<i>Richardia humistrata</i> (Cham. & Schltl.) Steud.	1	2	2			1			2							
	<i>Richardia stellaris</i> (Cham. & Schltl.) Steud.				1	1	1	2									
Solanaceae	<i>Solanum americanum</i> Mill.				1												
	<i>Solanum viarum</i> Dunal				1	1		3	3	3						1	1
Verbenaceae	<i>Glandularia selloi</i> (Spreng.) Tronc.	1															

**Appendix S2.** Results of randomization tests (MULTIV, Pillar 1999) comparing abundance, richness species, composition and seedling abundance according to life cycle and life forms. The symbol \* indicates significant differences ( $p \leq 0.05$ ) among the groups (forage allowance treatments/seasons).

Abundance			Richness	
Source of variation	Sum of squares (Q)	P-values *	Sum of squares (Q)	P-values *
<i>Between groups</i>	949.09	0.242	87.75	0.354
4% - 8%	30,625	0.953	27,563	0.354
4% - 12%	756.25	0.145	1	0.917
4% - 16%	110.25	0.528	45,563	0.187
8% - 12%	663.06	0.085	39,063	0.212
8% - 16%	76,563	0.521	2.25	0.728
12% - 16%	289	0.123	60,063	0.121
<i>Between groups</i>	12,999	0.001*	1333	0.001*
Spring - Summer	196	0.4	110.25	0.102
Spring - Autumn	2,376.6	0.002 *	420.25	0.004 *
Spring - Winter	10,920	0.002 *	1,225	0.001 *
Summer - Autumn	1,207.6	0.069	100	0.09
Summer - Winter	8,190.3	0.004 *	600.25	0.007 *
Autumn - Winter	3,108.1	0.001*	210.25	0.007 *
Composition				
Source of variation	Sum of squares (Q)	P-values *		
<i>Between groups</i>	1.8307	0.0004		
4% - 8%	0.48839	0.0828		
4% - 12%	0.72374	0.0014*		
4% - 16%	1.209	0.0013*		
8% - 12%	0.30111	0.177		
8% - 16%	0.56905	0.0637		
12% - 16%	0.37008	0.1047		
<i>Between groups</i>	6.2544	0.0001		
Spring - Summer	2.3784	0.0029		
Spring - Autumn	2.2278	0.001		
Spring - Winter	2.4689	0.0011		
Summer - Autumn	1.6214	0.0015		
Summer - Winter	2.0195	0.0016		
Autumn - Winter	1.7927	0.0017		
Perennials			Non-perennials	
Source of variation	Sum of squares (Q)	P-values *	Sum of squares (Q)	P-values *
<i>Between groups</i>	975.34	0.089	49,844	0.528
4% - 8%	64	0.619	45,563	0.085
4% - 12%	900	0.044*	75,625	0.716
4% - 16%	232.56	0.229	22,563	0.473
8% - 12%	484	0.062	16	0.451
8% - 16%	52,563	0.559	4	0.514

12% - 16%	217.56	0.065	4	0.971
<i>Between groups</i>	7736.6	0.001	695.59	0.001
Spring - Summer	150.06	0.348	6.25	0.725
Spring - Autumn	1156	0.005*	217.56	0.012*
Spring - Winter	6642.3	0.003*	529	0.002*
Summer - Autumn	473.06	0.172	150.06	0.006*
Summer - Winter	4795.6	0.006*	420.25	0.005*
Autumn - Winter	2256.3	0.002*	68,063	0.003*
<b>Grasses</b>		<b>Sedges and rushes</b>		
<b>Source of variation</b>	<b>Sum of squares (Q)</b>	<b>P-values *</b>	<b>Sum of squares (Q)</b>	<b>P-values *</b>
<i>Between groups</i>	41,344	0.591	949	0.019*
4% - 8%	2.25	0.844	132.25	0.328
4% - 12%	36	0.292	841	0.035*
4% - 16%	15,625	0.815	462.25	0.059*
8% - 12%	20.25	0.429	306.25	0.032*
8% - 16%	0.0625	1	100	0.329
12% - 16%	22,563	0.367	56.25	0.178
<i>Between groups</i>	2144.6	0.001*	4083.8	0.001*
Spring - Summer	451.56	0.009*	770.06	0.019*
Spring - Autumn	473.06	0.007*	30.25	0.504
Spring - Winter	2139.1	0.002*	1242.6	0.008*
Summer - Autumn	0.25	0.953	495.06	0.109
Summer - Winter	625	0.005*	3969	0.003*
Autumn - Winter	600.25	0.002*	1660.6	0.003*
<b>Herbs</b>		<b>Subshrubs/shrubs</b>		
	<b>Sum of squares (Q)</b>	<b>P-values *</b>	<b>Sum of squares (Q)</b>	<b>P-values *</b>
<i>Between groups</i>	155.59	0.057	15,938	0.55
4% - 8%	105.06	0.032*	1	0.317
4% - 12%	60,063	0.108	0.0625	1
4% - 16%	126.56	0.058*	1	0.271
8% - 12%	6.25	0.73	0.5625	0.49
8% - 16%	1	0.804	0	1
12% - 16%	12.25	0.719	0.5625	0.595
<i>Between groups</i>	1080.6	0.001	55,938	0.04
Spring - Summer	484	0.003*	15,625	0.297
Spring - Autumn	992.25	0.002*	1	0.312
Spring - Winter	517.56	0.003*	0.25	0.763
Summer - Autumn	90.25	0.007*	50,625	0.025*
Summer - Winter	0.5625	0.848	30,625	0.125
Autumn - Winter	76,563	0.001*	0.25	0.683

**Appendix S3.** Composition of species in dispersion or mature fruits in grazed subtropical grassland in southern Brazil, by season and forage allowance treatment (FA; see text for detail). The number of mature or dispersing reproductive structures produced from individual flowers (†), inflorescence (‡) or inflorescence branches (§).

Family	Species	Spring				Summer				Autumn				Winter			
		4%	8%	12%	16%	4%	8%	12%	16%	4%	8%	12%	16%	4%	8%	12%	16%
	<i>Justicia axillaris</i> (Nees) Lindau	†		1													
Acanthaceae	<i>Ruellia hypericoides</i> (Nees) Lindau	†									1						
	<i>Ruellia morongii</i> Britton	†	1				1				1						
Amaranthaceae	<i>Pfaffia tuberosa</i> (Spreng.) Hicken	‡	1					1									
Amaryllidaceae	<i>Zephyranthes</i> sp.	†					1										
	<i>Centella asiatica</i> (L.) Urb.	‡	1				1	1	3		1						
Apiaceae	<i>Eryngium ciliatum</i> Cham. & Schltdl.	§		2	1				1								
	<i>Eryngium horridum</i> Malme	§		2	2	1		1		1		3		2			
Aristolochiaceae	<i>Aristolochia sessilifolia</i> (Klotzsch) Duch.	†			1												
	<i>Aspilia montevidensis</i> (Spreng.) Kuntze	‡		1	2				1								
	<i>Baccharis crispa</i> Spreng.	‡			2			1	4	1	1	5	7	2		1	1
	<i>Baccharis riograndensis</i> Malag. & J.Vidal	‡		1					1								
	<i>Chaptalia exscapa</i> (Pers.) Baker	‡									2						1
	<i>Chaptalia integerrima</i> (Vell.) Burkart	‡	1														
	<i>Chaptalia piloselloides</i> (Vahl) Baker	‡									3						
	<i>Chaptalia runcinata</i> Kunth	‡									2		1				1
	<i>Chevreulia acuminata</i> Less.	‡			1												
	<i>Chevreulia sarmentosa</i> (Pers.) S.F.Blake	‡													3		
Asteraceae	<i>Chromolaena squarrosa</i> (Hook. & Arn.) R.M.King & H.Rob.	‡										2	1	2			1
	<i>Elephantopus mollis</i> Kunth	‡		1													
	<i>Gamochaeta americana</i> (Will.) Wedd.	‡	1	1	1												
	<i>Noticastrum calvatum</i> (Baker) Cuatrec.	‡											1				
	<i>Pterocaulon angustifolium</i> DC.	‡							1		1	4	4	2		1	1
	<i>Senecio brasiliensis</i> (Spreng.) Less.	‡	3								2				1		
	<i>Senecio leptolobus</i> DC.	‡	1	1		1											
	<i>Stenachaenium campestre</i> Baker	‡	1		1												
	<i>Vernonanthura nudiflora</i> (Less.) H.Rob.	‡			1		1		1		3	5	5	2		1	2
Convolvulaceae	<i>Evolvulus sericeus</i> Sw.	†	1				3										
Cyperaceae	<i>Abildgaardia ovata</i> (Burm.f.) Kral	§	3		1		1										

	<i>Bulbostylis subtilis</i> M.G. López	§	1						1										
	<i>Fimbristylis dichotoma</i> (L.) Vahl	§	2	1		2		1		2	3								1
	<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.	§								1									1
	<i>Cyperus sesquiflorus</i> (Torr.) Mattf. & Kük.	§									1								
	<i>Rhynchospora barrosiana</i> Guagl.	§		3	5	3		1	2	3								1	1
	<i>Rhynchospora edwalliana</i> Boeckeler	§						1											
	<i>Rhynchospora tenuis</i> Link	§		1	1	4		1		4								2	1
Euphorbiaceae	<i>Tragia bahiensis</i> Müll. Arg.	†	1		1	1			1										
	<i>Desmodium incanum</i> DC.	†	2	2							2							1	
	<i>Galactia marginalis</i> Benth.	†							1										
Fabaceae	<i>Macropodium prostratum</i> (Benth.) Urb.	†								1									
	<i>Pomaria pilosa</i> (Vogel) B.B. Simpson & G.P. Lewis	†																1	
	<i>Stylosanthes montevidensis</i> Vogel	†				1													1
	<i>Zornia</i> sp.	†	2					2											
Hypoxidaceae	<i>Hypoxis decumbens</i> L.	†						1	1		1	3	4	2				6	1
Iridaceae	<i>Sisyrinchium micranthum</i> Cav.	†	1																
	<i>Sisyrinchium vaginatum</i> Spreng.	†	1																
Linaceae	<i>Cliococca selaginoides</i> (Lam.) C.M. Rogers & Mildner	†	1					2											2
Lythraceae	<i>Cuphea glutinosa</i> Cham. & Schldtl.	†				1													
Melastomataceae	<i>Tibouchina gracilis</i> (Bonpl.) Cogn.	†		1					1			1							
	<i>Oxalis bipartita</i> A. St.-Hil.	†																3	1
Oxalidaceae	<i>Oxalis brasiliensis</i> G. Lodd.	†																	1
	<i>Oxalis eriocarpa</i> DC.	†									1							2	
	<i>Oxalis lasiopetala</i> Zucc.	†																1	
Plantaginaceae	<i>Mecardonia procumbens</i> var. <i>tenella</i> (Cham. & Schldtl.) V.C. Souza	§																	2
	<i>Andropogon lateralis</i> Nees	§		4	2	4		7	5	7		5	5	6					
	<i>Aristida filifolia</i> (Arechav.) Herter	§							2	3				2					
	<i>Aristida jubata</i> (Arechav.) Herter	§			3	3													
	<i>Aristida laevis</i> (Nees) Kunth	§		2	3	3		1	2	2			2	2					
Poaceae	<i>Aristida venustula</i> Arechav.	§								1									
	<i>Axonopus affinis</i> Chase <sup>2</sup>	§	4	1		2	4	3	3	5	6	1	1	2	6	2			
	<i>Chascolytrum rufum</i> J. Presl	§				1													
	<i>Chascolytrum subaristatum</i> (Lam.) Desv.	§		1	1	1													
	<i>Dichantherium sabulorum</i> (Lam.) Gould & C.A. Clark	§		3		2		3	2	1		5	2	3					
	<i>Eragrostis airoides</i> Nees	§										2		1					

	<i>Eragrostis lugens</i> Nees	§			1		1		1				
	<i>Eragrostis neesii</i> Trin.	§		1				1		1		1	
	<i>Eragrostis polytricha</i> Nees	§			1								
	<i>Mnesithea selloana</i> (Hack.) de Koning & Sosef	§	5	6	4	2	4	3	2	1	1		
	<i>Paspalum lepton</i> Schult.	§	1			1			1			1	
	<i>Paspalum notatum</i> Flügge	§	7	5	3	1	4	4	4	4	2	1	
	<i>Paspalum pauciciliatum</i> (Parodi) Herter	§	1				1	1					
	<i>Paspalum paucifolium</i> Swallen	§								2			
	<i>Paspalum plicatulum</i> Michx.	§	1	3	1	4		1	2	3	1	1	
	<i>Paspalum pumilum</i> Nees	§				1		2		1		1	
	<i>Piptochaetium montevidense</i> (Spreng.) Parodi	§	3	4	3	4		1	1	1			
	<i>Piptochaetium ruprechtianum</i> É.Desv.	§		1									
	<i>Piptochaetium stipoides</i> (Trin. & Rupr.) Hack. & Arechav.	§		1	2								
	<i>Schizachyrium condensatum</i> (Kunth) Nees	§									1	2	
	<i>Setaria parviflora</i> (Poir.) M. Kerguelen	§	4	6	3	6	4	5	5	4	4	6	8
	<i>Setaria vaginata</i> Spreng.	§		1		2					1	2	4
	<i>Sorghastrum setosum</i> (Griseb.) Hitchc.	§								1			
	<i>Sporobolus indicus</i> (L.) R.Br.	§		1			1			2	3	1	
	<i>Steinchisma hians</i> (Elliott) Nash	§	8	8	6	3	8	7	6	2	8	6	4
Polygalaceae	<i>Polygala australis</i> A.W. Benn.	‡											1
	<i>Borreria palustris</i> (Cham. & Schltdl.) Bacigalupo & E.L.Cabral	‡			1	1							
	<i>Borreria verticillata</i> (L.) G.Mey.	‡				1							
Rubiaceae	<i>Richardia grandiflora</i> (Cham. & Schltdl.) Steud.	‡		1		1			1		1		1
	<i>Richardia humistrata</i> (Cham. & Schltdl.) Steud.	‡											1
	<i>Richardia stellaris</i> (Cham. & Schltdl.) Steud.	‡	6	2	1	1	6	2	1	1			1
	<i>Spermacoce eryngioides</i> (Cham. & Schltdl.) Kuntze	‡	1		1			1			1		

## **CAPÍTULO 2: Flowering and fruiting phenology patterns in South Brazilian subtropical grasslands from a restoration perspective<sup>2</sup>**

**Running head:** Phenology as a guideline for restoration planning

**Title:** Flowering and fruiting phenology patterns in Southern Brazilian subtropical grasslands from a restoration perspective

Graziela Har Minervini Silva<sup>1,2,3</sup>, Luciana da Silva Menezes<sup>1</sup>, Fábio Piccin Torchelsen<sup>1</sup>, Daniel Dutra Saraiva<sup>1</sup>, Gerhard Ernst Overbeck<sup>1,2</sup>

<sup>1</sup>Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Botânica. Avenida Bento Gonçalves 9500, Porto Alegre, RS, Brasil.

<sup>2</sup>Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Botânica. Avenida Bento Gonçalves 9500, Porto Alegre, RS, Brasil.

<sup>3</sup>Corresponding address: graziela.minervini@gmail.com

**Author contributions:** GHMS, GEO conceived and designed the research. GHMS, LSM performed the field work, FPT, DDS performed the statistical analysis, GHMS analyzed the data and led the writing of the manuscript, GEO critically reviewed and edited the manuscript, all authors contributed to the final version of the manuscript.

---

<sup>2</sup> Este manuscrito será submetido à revista *Restoration Ecology* e está formatado conforme às normas da revista.



## **Abstract**

In tropical and subtropical grasslands, restoration is still at the beginning and restoration techniques still need scientific underpinning. The timing of seed maturity and dispersal is of high relevance to plan effective restoration using directly collected seed or hay transfer. Over a period of twelve months, we monthly evaluated flowering and fruiting in grasslands under different grazing intensities (high, moderate, low, very low, ungrazed) in subtropical Brazil. To estimate the effect of the seasonal variation on the phenology of functional groups (C3 grass, C4 grass, monocots, dicots, shrubs) we used circular statistics and indicator species analysis. The highest phenological activity was found in spring and autumn. Shrubs had the highest relative abundance for flowering and fruiting, and C4 grasses fruiting were the most important in terms of species number. The flowering peak of most functional groups occurred in October, except for C4 grasses and shrubs that peaked in February and November. Fruiting of C3 grasses peaked in December, C4 grasses and shrubs were expressive in April and dicots and monocots peaked in October. Grazing treatments differed considerably in phenological patterns for all species groups. Monocots and dicots herbs showed high activity in the high grazing intensity, C3 grasses presented highest peaks in moderate and low grazing intensities, while C4 grasses peaked in very low grazing and shrubs showed the highest peaks in ungrazed. Flowering and fruiting occurred throughout the year in our study system, with clear variation among functional groups in response to climatic and management patterns. Our results can guide seed collection in the field or vegetation cutting for hay transfer.

Key words: inflorescence, seeds, propagules, dispersal, hay transfer, grazed, C3 grasses, C4 grasses, circular analysis.

## **Implications for practice**

- Hay that is to be used for seed introduction in restoration of subtropical grassland must be collected at various times throughout the year, in order to cover a wide spectrum of species from the target communities.
- Data of fruiting peaks, together with data on species with a potential germination rate, can guide hay collection with greater chances of success in the restoration project.
- Standardization in the quantitative estimation of the reproductive structures of species in grassland phenology studies is a difficult task. Our method allowed an approximation of the number of seeds produced, at least within functional groups or plant families that share morphological structures.
- Further studies should focus on quantity and viability of seeds, on the appropriate volume of hay for hay-transfer and experiment with seed introduction at different times over a year to be able to evaluate transfer success of different species. Additionally, the need of preparation of the recipient site needs to be evaluated.

## **Introduction**

Restoration of grassland has become a major conservation concern worldwide. However, in tropical and subtropical regions, grassland restoration is still much at the beginning (Buisson et al., 2019) when compared to temperate grasslands for which techniques, e.g. for species introduction, are well established (Kiehl et al., 2010). One specific challenge is the lack of commercially available seeds (Buisson et al., 2019; Overbeck et al., 2013). Hay transfer, that is cutting of vegetation at a well-conserved grassland site at the moment when seeds of grassland plants are ripe and transfer of the dried or green biomass that contains the seeds to the restoration site, appears as an interesting alternative. Hay transfer has been proven to be effective in different northern-hemisphere grasslands, such as in calcareous grasslands in Central Europe (e.g. Bischoff et al., 2018; Kiehl and Wagner, 2006), Puzta grasslands in Hungary (Török et al., 2012) and Mediterranean steppe (Coiffait-Gombault et al., 2011). However, the few studies conducted so far to test this method in tropical and subtropical regions have shown little or no effect (e.g. Le Stradic et al., 2014; Thomas et al., 2019). This may have several reasons, including both environmental constraints on the restoration sites and factors related to the biology of the target species, such as presence of dormancy and special germination requirements, or low seed viability (Kolb et al., 2016; Le Stradic et al., 2014).

One important aspect of the biology of any plant species is its phenology, i.e. the timing of plant life-cycle events, such as flowering and fruiting (Stucky et al., 2018). For ecological restoration by hay transfer, the timing of seed maturity and seed dispersal is key information (Buisson et al., 2017): if hay is collected at a moment with low or no seed availability, it obviously will not contribute to vegetation recovery. The study of phenological patterns, and especially of the timing of seed maturity, thus is of high relevance to plan effective restoration by hay transfer.

In temperate grasslands, flowering and fruiting may have high synchrony on the community level (Martínková et al., 2002). For ecological restoration, this means that one single cutting data can be used to transfer a large part of target species (Bischoff et al., 2018). In contrast, tropical and subtropical systems are found under contrasting climates, with different types of seasonality (e.g. Fetzl et al., 2017) and distinct phenological patterns that can differ much from those in temperate ecosystems. The subtropical grasslands in southern Brazil are composed of species with very contrasting phenological rhythms, evidenced, for instance, by the coexistence of C3 and C4 grasses (Andrade et al. 2019). However, detailed studies on the reproductive phenology of these systems are still missing. The few previous phenological studies in grasslands in the region (Oleques et al., 2017; Pinheiro et al., 2008) focused on interactions between plants and pollinators but did not consider grasses as these do not provide floral resources. For purposes of ecological restoration, this is a considerable limitation: grasses are the dominant species in these grasslands and can be considered important target species for restoration as they drive grassland dynamics and functionality. Importantly, in grasslands under grazing, cattle modulates species composition by consuming biomass, trampling and also contribution to seed dispersal (e.g. Boavista et al., 2019; Minervini Silva and Overbeck, 2021), which may also be made useful from a restoration perspective: Fedrigo et al. (2018) showed how grazing deferment, i.e. the removal of all livestock from a pasture for an period of time, contributed to quick recovery of overgrazed grassland in terms of species composition. Due to their effects on species composition and due to consumption of inflorescences by cattle, differences in grazing pressures thus may also influence seed production patterns.

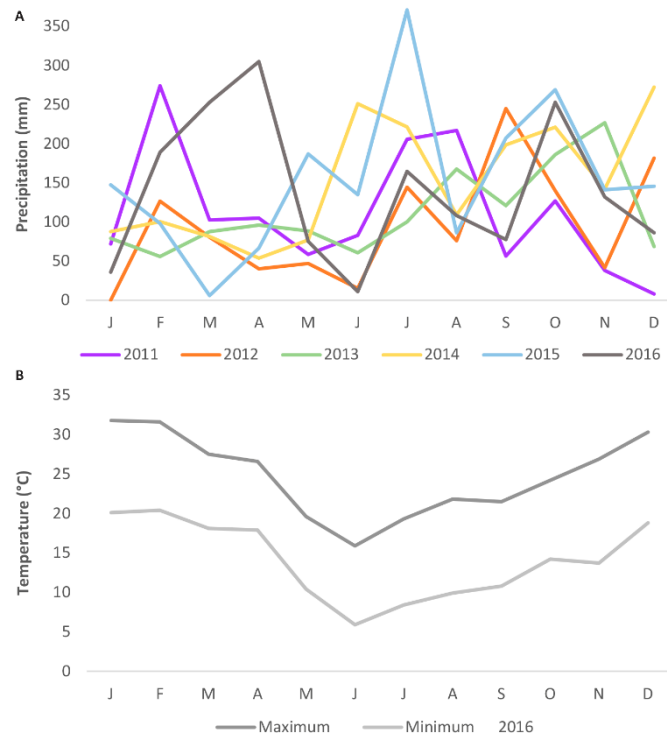
The aim of this study was to evaluate phenological patterns in grazed grasslands in southern Brazil from a restoration perspective, with a special focus on the timing of seed maturity and dispersal. To that end, we conducted monthly evaluations of the phenological stages of grassland species in grazed and ungrazed grasslands, over a period of twelve months.

We expected to find a long period of flowering and fruiting over the year, with peaks in spring and summer, associated with the phenological patterns of two major groups of grassland species, C3 and C4 grasses, but also for other species groups. Regarding grazing intensity, due to distinct forage allowances and selective grazing by cattle, we expect to observe differences in flowering and fruiting abundance and species composition among treatments.

## **Materials and methods**

### *Study site*

The study was performed in an experimental area of the Universidade Federal do Rio Grande do Sul in Eldorado do Sul, RS, Brazil (30°06'S and 51°41'W). Climate in the region is subtropical humid (Köppens' Cfa) with hot summers, mild to cool winters and no dry season (Alvares et al., 2013). During the study period (January to December 2016) total annual precipitation was 1,690mm (Fig. 1A) and average temperatures range from 14°C to 24.7°C (Data provided by the Weather Station of the UFRGS Agronomic Experimental Station) (Fig. 1B).



**Figure 1.** Monthly precipitation from 2001 to 2016 (A) and mean temperatures for each month in the study period (January to December 2016) (B), for the experimental site in Eldorado do Sul, RS, Brazil. Data provided by the Weather Station of the Agronomic Experimental Station of UFRGS.

The dominant vegetation type in the region is grassland characterized by the co-existence of C3 and C4 grasses (Overbeck et al., 2007). Poaceae, Asteraceae, Fabaceae, Cyperaceae, Rubiaceae and Apiaceae are the most important families in terms of species number (Andrade et al. 2019). The data were collected in an experimental area that is held with different levels of grazing intensity and thus forage allowances (FA) since 1986. The treatments are daily forage allowances of 4, 8, 12, and 16 kg of dry biomass (DM) per 100 kg of animal live weight (LW), where 4% represents the highest grazing intensity and 16% the lowest grazing intensity (for details see also Cruz et al., 2010; Fischer et al., 2019). Put simply: where grazing intensity is high, forage allowance is kept low due to high consumption rates by cattle. Biomass development is monitored monthly and stocking rates adjusted accordingly to

maintain the forage levels over the year. We also analyzed an adjacent ungrazed area which had not been grazed by cattle over ten years.

### *Phenological survey of vegetation*

A phenological survey of the vegetation, with monthly observations was realized from January to December 2016. To sample phenophases of grasslands plants, we selected 10 permanent plots of 1 m<sup>2</sup> in each grazing treatment and in the ungrazed area. The minimum distance between plots was 50 m, and they were situated at a minimum of 20 m from fences (adjacent treatments). All species with reproductive structures at the moment of sampling were recorded, using four phenophases: (1) presence of buds, (2) presence of flowers, (3) presence of mature fruits and (4) fruits in the phase of seed liberation. For analyses, we grouped phenophases into (a) buds and flowers (phenophases 1 and 2 “flowering”); and (b) mature fruits and fruits in the phase of seed liberation (phenophases 3 and 4: “fruiting”).

Quantification of reproductive structures differed among plant families due to the high diversity of inflorescence types with largely divergent flower types and numbers. We counted the number of reproductive structures arising from individual flowers, inflorescences (e.g. Asteraceae capitula) or inflorescence branches (e.g. Poaceae and Cyperaceae panicles) (Table S1). Each of the exemplified reproductive structures was considered as a unit. While this is a simplification, it does allow for a rough overall quantification, even though more detailed comparisons can only be done within each group of plants (for example, within Poaceae, within Asteraceae). Scientific names of the species and families follow Flora do Brasil 2020 (Flora do Brasil 2020).

### *Data analysis*

To characterize the community identified in the phenological survey, all species were classified according to the following functional groups: C3 grasses, C4 grasses, monocotyledonous herbs (excluding grasses; in the following: monocots), dicotyledonous herbs (in the following: dicots) and shrubs/subshrubs (in the following: shrubs).

To estimate the seasonal variation on flowering and fruiting of functional groups, we used circular statistics, a technique widely used in phenology studies (for more details see review by Morellato et al. 2010). In the circular analysis the year is represented as a circle, where January corresponds  $0^\circ$  or  $360^\circ$ . The analysis allows the visualization of production peaks in the flowering and fruiting stages throughout the year. We performed the circular analysis on the R environment (R Core Team, 2019), using the package ‘circular’ (Agostinelli and Lund, 2011) and the package ‘plotrix’ (Lemon et al., 2016). For the circular analysis we used the abundance of reproductive structures of functional groups (total structures for each functional group in each month) as a quantitative variable, considering the time factor (months) and the grazing factor (grazing treatment: 4 grazing levels and ungrazed). The first step to perform the circular analysis was to transform the variables into frequency (% , percentage of total structures for each functional group for each month). Then, we transformed the data (combining the angles with the variables) into radians to convert the data into circular (Zar, 2010). The results of the Watson test and the Rayleigh test (Zar, 2010) showed that the variables did not present circular normality and uniformity. Therefore, we used a non-parametric test (Watson-Wheeler test according to Zar, 2010) to compare circular variables of functional groups among the months and among the grazing intensity treatments.



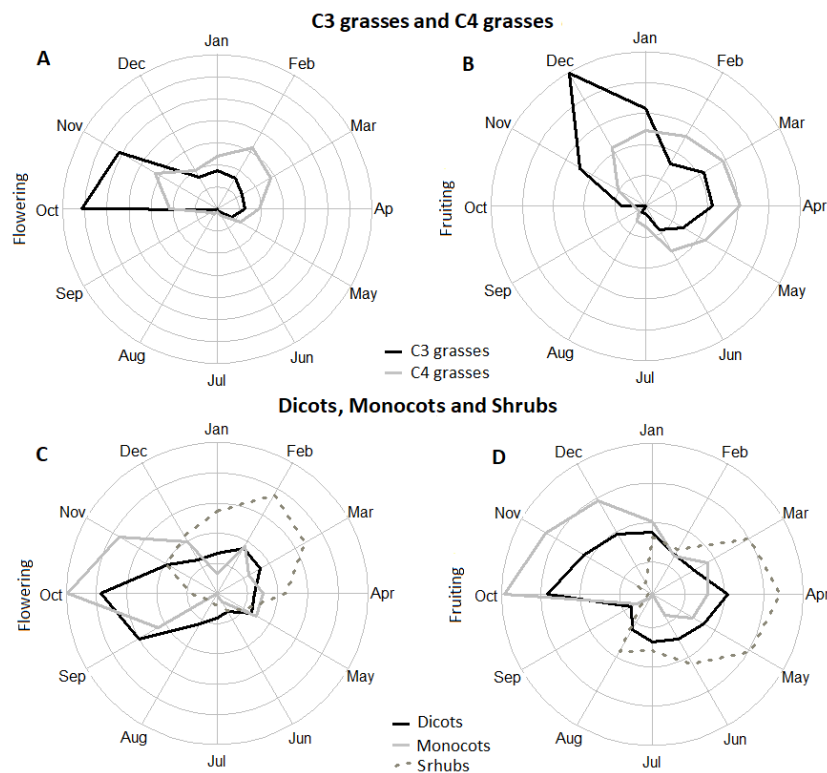
## Results

A total of 202 taxa were recorded in the phenological survey, distributed in 35 botanical families. The most important families regarding species richness and abundance of reproductive structures, considering all phenophases together, were Poaceae (44% of species; 21% of abundance of reproductive structures), Asteraceae (18% of species; 41% of abundance), Cyperaceae (7% of species; 11 % of abundance) and Apiaceae (4% of species; 13% of abundance). *Baccharis crispera*, *Eryngium horridum*, *Rhynchospora tenuis*, *Vernonanthura nudiflora*, *Austroeupatorium inulaefolium* and *Andropogon lateralis* were the dominant species with reproductive activity in terms of absolute abundance. Asteraceae presented the highest percentage of abundance considering all phenophases, while Poaceae was the most prominent family regarding richness. Shrubs had the highest relative abundance, in comparison with other functional groups, both in flowering and fruiting phases (Fig. 2). Dicots in the flowering phase (highest peak in October) and C4 grasses in the fruiting phase (highest peak in April) were the most representative functional groups in relation to species richness.

### *Phenology characteristics of functional groups in relation to seasonality*

The highest phenological activity was found in spring and autumn, as expected. In circular histograms, we observed that the flowering phase of C3 grasses peaked in October and gradually declined until January (Fig. 2A). The fruiting phase of C3 grasses had its highest levels in December (Fig. 2B). The flowering of C4 grasses peaked in February, stopped completely in southern hemisphere winter (June-August), and peaked again in November (Fig. 2A). Fruiting of C4 grasses was expressive from January to April, with a peak in March (Fig. 2B). The Watson-wheeler test comparing C3 and C4 grasses pointed significant different period of flowering ( $W=16.529$ ,  $df=2$ ,  $P=0.0003$ ) and fruiting ( $W=10.775$ ,  $df=2$ ,  $P=0.005$ ) between the two functional groups. Dicots showed peaks for flowering (Fig. 2C) and fruiting (Fig. 2D)

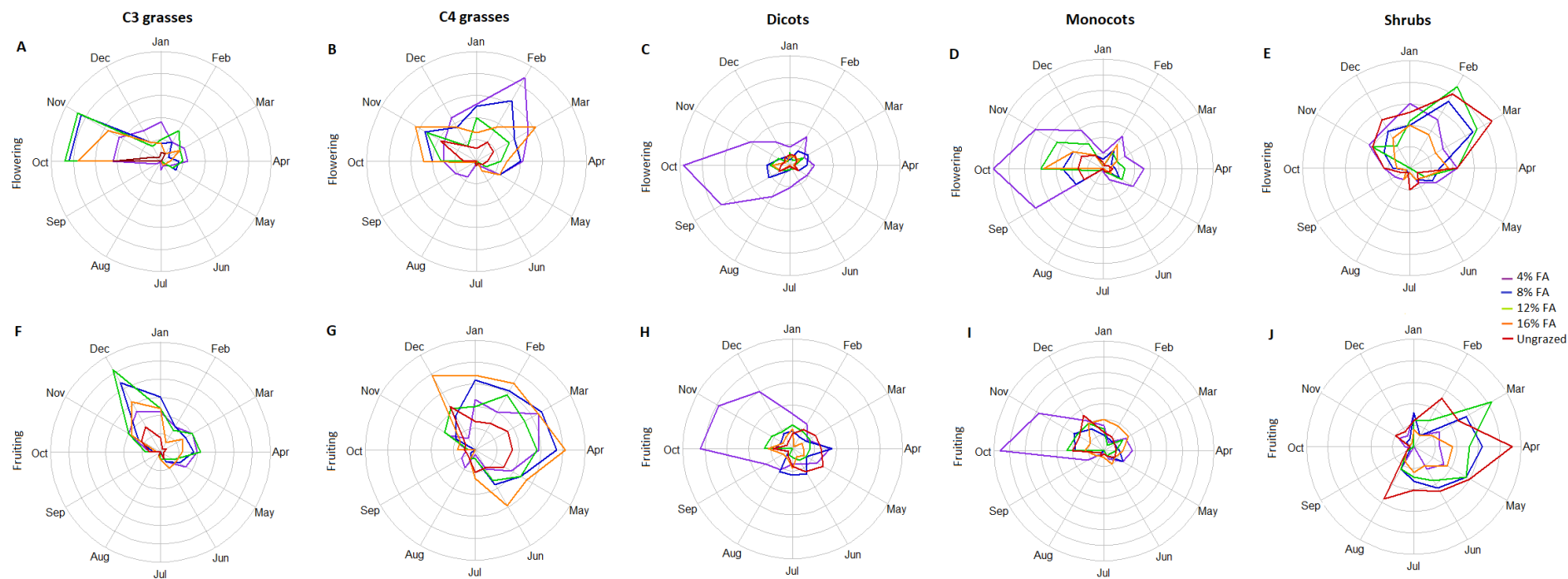
in October, but with rather high activity in November and December. Monocot flowering peaked in October (Fig. 2C) and fruiting from October to December (Fig. 2D). Shrubs were the most prominent group in terms of abundance of reproductive structures, with flowering peaks in February (Fig. 2C), and in April for fruiting phase (Fig. 2D). The Watson-wheeler test comparing dicots, monocots and shrubs showed significant differences of flowering phase ( $W=50.065$ ,  $df=4$ ,  $P<0.001$ ) and fruiting phase ( $W=48.182$ ,  $df=4$ ,  $P<0.001$ ) among the three functional groups.



**Figure 2.** Circular histograms representing functional groups frequencies (% , i.e. total percentage of structures for each month) for flowering and fruiting phenophases for the the studied subtropical grassland site in Eldorado do Sul, RS, Brazil. Circular histograms present the peaks over the year of functional groups (C3 grasses and C4 grasses (A-B); Dicots, Monocots and Shrubs (C-D), represented in the histogram legend) for flowering and fruiting.

### *Seasonal variation of flowering and fruiting in relation to grazing intensities*

For all species groups, grazing intensity had considerable influence on phenological patterns. High grazing intensity (4% FA) was associated to the highest reproductive activity of monocots and dicots. C3 grasses had highest peaks of flowering and fruiting under moderate (8% FA) and low (12% FA) grazing intensities; flowering was especially pronounced in October and November (Fig. 3A). Flowering and fruiting of C3 grasses in the ungrazed area were scarce, while we observed a later flowering peak under high grazing intensity (4% FA) (Fig. 3A) and low fruiting (Fig. 3F) compared to the other grazing treatments. C4 grasses showed the highest flowering peaks in February under high and moderate grazing intensities (4% FA and 8% FA, respectively: Fig. 3B). In November and March, the treatment of 16% FA (very low grazing intensity) also showed peaks for C4 grasses in the flowering, although to a lesser extent when compared to February (Fig. 3B). For the fruiting of C4 grasses, 16% FA (very low grazing intensity) was the treatment with the highest peaks, mainly in December, but peaks also occurred in April and June (Fig. 3G). The 4% FA treatment (high grazing intensity) had the highest peaks of dicots and monocots flowering (Fig. 3C-3D) and fruiting (Fig. 3H-3I), from September to December. The ungrazed area and the paddocks with 12% FA (low grazing intensity) showed the highest peaks of shrubs flowering and fruiting (Fig. 3E-3J). However, shrubs flowering and fruiting presented considerable variation among other treatments, with the low grazing treatment (12% FA) showing highest values in some months. The Watson-Wheeler test showed significant differences in flowering phases among grazing intensities for C3 grasses ( $W=15.559$ ,  $df=8$ ,  $P=0.049$ ), dicots ( $W=39.516$ ,  $df=8$ ,  $P<0.001$ ) and shrubs ( $W=14.761$ ,  $df=8$ ,  $P=0.015$ ). Fruiting, in contrast, was distinct among grazing intensities for dicots ( $W=71.559$ ,  $df=8$ ,  $P<0.001$ ) and monocots ( $W=25.52$ ,  $df=8$ ,  $P=0.001$ ).



**Figure 3.** Circular histograms representing functional group frequencies (% , i.e. total percentage of structures for each month) for flowering and fruiting) phenophases for the studied subtropical grassland site in Eldorado do Sul, RS, Brazil. Circular histograms present the peaks over the year of functional groups (C3 grasses A-F; C4 grasses B-G; Dicots C-H; Monocots D-I; Shrubs -E) for different grazing intensity treatments (% values FA: forage allowance) and the ungrazed treatment (i.e. no grazed by cattle site): purple: 4% FA; blue: 8% FA; green: 12% FA; orange: 16% FA; red: ungrazed).

## **Discussion**

### *Phenological patterns of subtropical grazed grasslands and implications for restoration*

Our study is the first to investigate the flowering and fruiting patterns of vegetation in Brazil's subtropical grasslands under different grazing intensities over the period of one year. Importantly – and in contrast to the existing phenological studies for the region (e.g. Pinheiro et al. 2008, Oleques et al. 2017) – we included grasses, which constitute an important food source for many animals: grazing animals such as cattle, but also granivorous birds (Gonnet, 2001; Zarco et al., 2019), rodents (Lucero and Callaway, 2018) or even insects (Linabury et al., 2019). Our results of grassland fruiting over time are of high relevance for ecological restoration, as they can guide seed collection in the field (e.g. Clinebell 2005) or vegetation cutting for hay transfer (e.g. Bischoff et al. 2018).

We observed that flowering and fruiting in our study system occur throughout the year, with clear variation in response to seasonal climate. Southern hemisphere winter clearly appeared – as expected – as the season with the least flowering and fruiting, and thus with low seed dispersal. The other seasons showed high flowering and fruiting activity, with different functional groups displaying different patterns. Our results have implications for restoration: whether seeds are to be introduced at a restoration site by hay transfer or after direct collection in the field, harvesting at the donor site should be realized at several dates if a large part of the species of the plant community were to be considered. Exact dates for cutting will always have to be defined based on the observation of the phenophases in the year in question: it is well known that climatic variation between years can influence population biology of grasses (e.g. Peters 2000) and thus exact timing of seed production. At any rate, our results can serve as a general basis for restoration planning.

Standardization in the quantitative estimation of the reproductive structures of species in grassland phenology studies is a challenge important not only to indicate phenological

activity, but also to quantify seed production (Le Stradic et al., 2018). Given that in many plant families not all flowers develop into fruits, or that many seeds are not viable (e.g. in grasses: Campbell, 1985), it should be noted that our approach cannot give precise information about seed production in quantitative terms. While the method used for counting the reproductive structures used by us certainly is a simplification, it does allow an approximation, at least within functional groups or plant families that share morphological structures. The quantification of seed production appears as important next research step, alongside with experimentation of how management can be used to increase seed production.

#### *Phenological characteristics of functional groups in relation to seasonality*

We verified high synchronism among species within functional group. Díaz et al. (1994) observed that species in Argentinean temperate grasslands with the same growth form had similar temporal patterns of flowering and fruiting, with grasses with below ground reserves very early and annuals (in their majority herbs) very late. In our study, dicot flowering and fruiting peaked in October, that is, mid-spring, and C3 and C4 grasses had their flowering peaks in October and November and fruiting peaks in December and April, respectively.

Other phenology studies in grasslands communities have shown that phenological patterns can be related to the weather regime, to management (grazed/ungrazed) or to soil conditions, or even combinations of these factors (Leon & Bertiller 1982; Bergmeier & Matthäs 1996; Han et al. 2015; Zhu et al. 2016; Li et al. 2019). In our study, we identified flowering and/or fruiting peaks during periods of higher rainfall (February to April and October to November). In a study on the phenology between stony and sandy *campo rupestre* grassland communities, the higher production of flowers and fruits in sandy grasslands was considered to be potentially related to greater water retention in the soil, indicating that soil content may restrict the reproductive activity in Neotropical grasslands (Le Stradic et al., 2018).

In our study, shrubs showed the highest relative abundance, both in flowering and fruiting, compared to the other functional groups. This is explained by the high contribution of Asteraceae species (especially species of the Eupatorieae tribe and several *Baccharis* species) which show high investment in the production of reproductive structures, with flowering and fruiting peaks between the end of summer (March) and autumn (May), respectively (e.g. Beretta et al. 2008).

#### *Seasonal variation of flowering and fruiting in relation to grazing intensities*

We observed different responses of functional groups to grazing management. C3 and C4 grasses showed later flowering in the treatments with high (4% FA) and moderate (8% FA) grazing intensities in relation to the other treatments. Under grazing conditions, some species can advance flowering to ensure their survival by sexual reproduction. Further, higher exposition to sunlight and higher temperature in gaps caused by strong grazing may also lead to advanced flowering (Li et al. 2019). Other plants delay their flowering in order to invest in the maintenance of vegetative parts and obtain compensatory growth of the leaves by increasing the light phase of photosynthesis (Lennartsson et al., 2012; Li et al., 2019). Consumption of plant biomass and trampling by cattle can also a delay of flowering (Li et al., 2019). Mechanisms related to palatability likewise can influence timing of flowering, delaying the sexual reproductive phase as adapting the plant against herbivory, or advancing flowering to seed production as a strategy for dispersal by zoochory (Janzen, 1984; Leon and Bertiller, 1982).

In our study, flowering and fruiting were reduced in ungrazed areas when compared to grazed sites, especially for C3 grasses and dicots. On the other hand, flowering and fruiting of shrubs were high in the ungrazed areas. For our study region, it has been shown that cessation of grazing leads, within few years, to the dominance of tall-growing tussock grasses and litter

accumulation, and a strong decrease in species richness (Ferreira et al., 2020; Lezama et al., 2014). The exclusion of grazing for longer periods benefits shrubs (Oliveira & Pillar 2004), a highly competitive functional group in terms of high vegetation cover and high investment in sexual reproduction. Grassland under long periods of grazing exclusion thus appears to be less interesting as a donor site for hay transfer, as seed content of typical grassland species likely is low, especially when related to biomass volume.

In South Brazilian grasslands, overgrazing does not necessary lead to reduced species richness at the plot scale, but to overall homogenization of the plant community (Fedrigo et al. 2018). Flowering and fruiting of C3 grasses was reduced in the high grazing intensity (4% FA) study plots, while dicots and monocots showed intensive flowering over extended periods. C4 grasses showed the highest peak of flowering in high grazing intensity (4% FA), due to the high presence of low-growing grasses (such as *Axonopus affinis* and *Paspalum* ssp.) that were common at heavily grazed sites. However, the C4 grasses fruiting was lower in 4% FA compared to other grazing intensities, probably due to the consumption of inflorescences by cattle, stimulated by low forage allowance and the investment of these species in endozoochory as a dispersal strategy (Minervini Silva and Overbeck, 2021). Also, vegetation height is lower in treatments with high grazing intensity (Oleques et al., 2019), turning heavily grazed sites unattractive as doner sites for hay transfer. However, grazing deferments, i.e. rest periods, may not only promote to quick recovery of the plant community (Fedrigo et al., 2018), but also increase flowering (Han et al., 2015; Zhu et al., 2016).

### *Conclusions and perspectives*

In our study system, flowering and fruiting occurred throughout the year. Therefore, hay used for purpose of seed introduction must be collected at various times throughout the year in order to cover a wide spectrum of species from the target communities in subtropical grasslands.



While exact timing of hay transfer certainly will vary among specific sites and also years, the data presented here is only a first step towards the definition of hay transfer protocols for grasslands in the region that aim at cost-effective biodiversity recovery (Palma & Laurance 2015). Further studies should focus on quantity and viability of seeds, and also on the appropriate volume of hay or amount of seed to be harvested and transferred at different dates. Information on germination potential and changes in germination rates over time also will be of importance, just as tests on the best preparation of the recipient sites to increase establishment. Studies in temperate grasslands in Europe report that green hay transfer (i.e. fresh hay collected from ‘donor’ site to a nearby ‘receptor’ site) tends to enhance plant establishment (Albert et al., 2019; Wagner et al., 2020). However, it is not clear if this is also true for tropical and subtropical systems. Monitoring of plant establishment will indicate if an additional sowing of hand-collected seed of species underrepresented or absent in the hay might be necessary to maximize establishment of a higher range of species (Wagner et al., 2020). The restoration of tropical and subtropical grasslands is still a challenge, but likely will advance by further studies that help to close knowledge gaps related to efficient restoration techniques, priority species and their biology.

### **Acknowledgements**

We thank members of the Laboratory for the Study of Grassland Vegetation (Levcamp), UFRGS, for assistance in field and greenhouse work and in plant identification, especially Lucas Pinheiro, Kassiane Helmicki, Helena Streit, Lukiél Oliveira, Mariana Vieira, Dióber Lucas, Bianca Andrade, Ingmar Staude, Juliana Schaefer, Pedro Silva Filho and Ilsi Boldrini. This study was financed in part by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) research grant to GEO (477618/2013-8) and in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. GEO

acknowledges CNPq productivity grant (310022/2015-0 and 310345/2018-9) and GHMS acknowledges CAPES scholarship.

## References

- Adler, P., Raff, D., Lauenroth, W., 2001. The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*. <https://doi.org/10.1007/s004420100737>
- Agostinelli, C., Lund, U., 2011. R package circular: Circular Statistics (version 0.4-3). CA Dep. Environ. Sci. Informatics Stat. Ca' Foscari Univ. Venice, Italy. UL Dep. Stat. Calif. Polytech. State Univ. San Luis Obispo, California, USA.
- Albert, Á.J., Mudrak, O., Jongepierova, I., Fajmon, K., Frei, I., ˇSevcikova, M., Klimešova, J., Doležal, J., 2019. Grassland restoration on ex-arable land by transfer of brush-harvested propagules and green hay. *Agric. Ecosyst. Environ.* 272, 74–82. <https://doi.org/10.1016/j.agee.2018.11.008>
- Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013. Koppen's climate classification map for Brazil. *Meteorol. Zeitschrift* 22, 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecol. Monogr.* <https://doi.org/10.1890/12-2010.1>
- Andrade, B.O., Bonilha, C.L., Overbeck, G.E., Velez-Martin, E., Rolim, R.G., Bordignon, S.A.L., Schneider, A.A., Vogel Ely, C., Lucas, D.B., Garcia, E.N., dos Santos, E.D., Torchelsen, F.P., Vieira, M.S., Silva Filho, P.J.S., Ferreira, P.M.A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V.D., Boldrini, I.I., 2019. Classification of South Brazilian grasslands: Implications for conservation. *Appl. Veg. Sci.* 22, 168–184. <https://doi.org/10.1111/avsc.12413>
- Arruda, A.J., Buisson, E., Poschlod, P., Silveira, F.A.O., 2018. How have we studied seed rain in grasslands and what do we need to improve for better restoration? *Restor. Ecol.* <https://doi.org/10.1111/rec.12686>
- Baker, H.G., 1989. Some Aspects of the Natural History of Seed Banks, in: *Ecology of Soil Seed Banks*. pp. 9–21. <https://doi.org/10.1016/b978-0-12-440405-2.50007-5>
- Bazzaz, F.A., 1983. Characteristics of populations in relation to disturbance in natural and man-modified ecosystems., in: Mooney, H.A., Godron, M. (Eds.), *Disturbance and Ecosystems*. Berlin, pp. 259–275. [https://doi.org/10.1007/978-3-642-69137-9\\_17](https://doi.org/10.1007/978-3-642-69137-9_17)
- Benke, C.S.C., Morellato, L.P.C., 2002. Comparaçao de dois metodos de avaliaçao da fenologia de plantas, sua interpretaçao e representaçao. *Rev. Bras. Botanica* 25, 269–275. <https://doi.org/10.1590/s0100-84042002000300003>
- Beretta, M.E., Fernandes, A.C., Schneider, A.A., Ritter, M.R., 2008. A familia Asteraceae no Parque Estadual de Itapua, Viamao, Rio Grande do Sul, Brasil. *Rev. Bras. Biociencia* 6, 189–216.
- Bergmeier, E., Matthas, U., 1996. Quantitative studies of phenology and early effects of non-grazing in Cretan phrygana vegetation. *J. Veg. Sci.* 7, 229–236. <https://doi.org/10.2307/3236323>
- Bischoff, A., Hoboy, S., Winter, N., Warthemann, G., 2018. Hay and seed transfer to re-

- establish rare grassland species and communities: How important are date and soil preparation? *Biol. Conserv.* 221, 182–189.  
<https://doi.org/https://doi.org/10.1016/j.biocon.2018.02.033>
- Boavista, L. da R., Trindade, J.P.P., Overbeck, G.E., Müller, S.C., 2019. Effects of grazing regimes on the temporal dynamics of grassland communities. *Appl. Veg. Sci.* 22, 326–335. <https://doi.org/10.1111/avsc.12432>
- Boldrini, I.I., Longhi-Wagner, H.M., Boechat, S. de C., 2008. *Morfologia e taxonomia de gramíneas Sul-Rio-Grandenses*, 2 nd. ed. UFRGS Editora, Porto Alegre.
- Bond, W.J., Keeley, J.E., 2005. Fire as a global “herbivore”: The ecology and evolution of flammable ecosystems. *Trends Ecol. Evol.* 20, 387–394.  
<https://doi.org/10.1016/j.tree.2005.04.025>
- Buisson, E., Alvarado, S.T., Le Stradic, S., Morellato, L.P.C., 2017. Plant phenological research enhances ecological restoration. *Restor. Ecol.* 25, 164–171.  
<https://doi.org/10.1111/rec.12471>
- Buisson, E., Le Stradic, S., Silveira, F.A.O., Durigan, G., Overbeck, G.E., Fidelis, A., Fernandes, G.W., Bond, W.J., Hermann, J.-M., Mahy, G., Alvarado, S.T., Zaloumis, N.P., Veldman, J.W., 2019. Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. *Biol. Rev.* 94, 590–609.  
<https://doi.org/10.1111/brv.12470>
- Bullock, J.M., Moy, I.L., 2004. Plants as seed traps: Inter-specific interference with dispersal. *Acta Oecologica*. <https://doi.org/10.1016/j.actao.2003.10.005>
- Burkart, A.E., 1969. *Flora ilustrada de Entre Rios (Argentina)*, Instituto Nacional de Tecnología Agropecuaria. Buenos Aires.
- Campbell, C.S., 1985. The subfamilies and tribes of Gramineae (Poaceae) in the southeastern United States. *J. Arnold Arboretum.* 66, 123–199. <https://doi.org/10.5962/bhl.part.13181>
- Cantero, J.J., Partel, M., Zobel, M., 1999. Is Species Richness Dependent on the Neighbouring Stands? An Analysis of the Community Patterns in Mountain Grasslands of Central Argentina. *Oikos* 87, 346–354. <https://doi.org/10.2307/3546750>
- Chabrierie, O., Alard, D., 2005. Comparison of three seed trap types in a chalk grassland: Toward a standardised protocol. *Plant Ecol.* <https://doi.org/10.1007/s11258-004-0024-2>
- Clinebell, R.R., 2005. Tips for gathering individual species, in: Stephen Packard, C.F.M. (Ed.), *The Tallgrass Restoration Handbook*. Island press, pp. 127–134.
- Coiffait-Gombault, C., Buisson, E., Dutoit, T., 2011. Hay Transfer Promotes Establishment of Mediterranean Steppe Vegetation on Soil Disturbed by Pipeline Construction. *Restor. Ecol.* 19, 214–222. <https://doi.org/10.1111/j.1526-100X.2010.00706.x>
- Cosyns, E., Claerbout, S., Lamoot, I., Hoffmann, M., 2005. Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecol.* 178, 149–162.  
<https://doi.org/10.1007/s11258-004-2846-3>
- Cruz, P., De Quadros, F.L.F., Theau, J.P., Frizzo, A., Jouany, C., Duru, M., Carvalho, P.C.F., 2010. Leaf traits as functional descriptors of the intensity of continuous grazing in native grasslands in the South of Brazil. *Rangel. Ecol. Manag.* 63, 350–358.  
<https://doi.org/10.2111/08-016.1>
- da Silva Menezes, L., Vogel Ely, C., Lucas, D.B., Minervini Silva, G.H., Boldrini, I.I., Overbeck, G.E., 2018. Plant species richness record in Brazilian Pampa grasslands and implications. *Rev. Bras. Bot.* 41, 817–823. <https://doi.org/10.1007/s40415-018-0492-6>

- de Souza Vieira, M., Overbeck, G.E., 2020. Small seed bank in grasslands and tree plantations in former grassland sites in the South Brazilian highlands. *Biotropica*. <https://doi.org/10.1111/btp.12785>
- Deble, L.P., Marchiori, J.N.C., 2014. Sinopse do gênero *Gamochaeta* Weddel (Asteraceae-Gnaphalieae) no Brasil. *Balduinia* 0, 21–31. <https://doi.org/10.5902/2358198014040>
- Díaz, S., Acosta, A., Cabido, M., Diaz, S., 1994. Grazing and the Phenology of Flowering and Fruiting in a Montane Grassland in Argentina: A Niche Approach. *Oikos* 70, 287. <https://doi.org/10.2307/3545640>
- Duarte, E., Aona-Pinheiro, Lidyanne Costa, G., Bezerra, C., Soares de Oliveira Neto, J., 2019. Glossário eletrônico de morfologia vegetal: sementes e propágulos [WWW Document]. Univ. Fed. Góias/ Univ. Fed. do Recôncavo da Bahia. URL [www.projetosbotanica.icb.ufg.br/guia\\_sd/glossary](http://www.projetosbotanica.icb.ufg.br/guia_sd/glossary) (accessed 11.18.20).
- Fedrigo, J.K., Ataíde, P.F., Filho, J.A., Oliveira, L. V., Jaurena, M., Laca, E.A., Overbeck, G.E., Nabinger, C., 2018. Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. *Restor. Ecol.* 26, 677–685. <https://doi.org/10.1111/rec.12635>
- Feldman, S.R., Alzugaray, C., Lewis, J.P., 2007. Relación entre la vegetación y el banco de semillas de un espartillar de *Spartina argentinensis*. *Cienc. e Investig. Agrar.* 34, 41–48. <https://doi.org/10.4067/s0718-16202007000100005>
- Ferreira, P.M.A., Andrade, B.O., Podgaiski, L.R., Dias, A.C., Pillar, V.D., Overbeck, G.E., Milton de, S.M., Boldrini, I.I., 2020. Long-term ecological research in southern Brazil grasslands: Effects of grazing exclusion and deferred grazing on plant and arthropod communities. *PLoS One* 15, 1–23. <https://doi.org/10.1371/journal.pone.0227706>
- Fetzel, T., Havlik, P., Herrero, M., Erb, K.H., 2017. Seasonality constraints to livestock grazing intensity. *Glob. Chang. Biol.* 23, 1636–1647. <https://doi.org/10.1111/gcb.13591>
- Fidelis, A., Blanco, C., 2014. Does fire induce flowering in Brazilian subtropical grasslands? *Appl. Veg. Sci.* 17, 690–699. <https://doi.org/10.1111/avsc.12098>
- Fischer, F.M., Bonnet, O.J.F., Cezimbra, I.M., Pillar, V.D., 2019. Long-term effects of grazing intensity on strategies and spatial components of functional diversity in subtropical grassland. *Appl. Veg. Sci.* 22, 39–47. <https://doi.org/10.1111/avsc.12402>
- Flora do Brasil 2020, 2020. Flora do Brasil 2020 [WWW Document]. Jard. Botânico do Rio Janeiro. URL [www.floradobrasil.jbrj.gov.br](http://www.floradobrasil.jbrj.gov.br) (accessed 11.12.20).
- Funes, G., Basconcelo, S., Díaz, S., Cabido, M., 2001. Edaphic patchiness influences grassland regeneration from the soil seed-bank in mountain grasslands of central Argentina. *Austral Ecol.* 26, 205–212. <https://doi.org/10.1046/j.1442-9993.2001.01102.x>
- Galíndez, G., Ortega-Baes, P., Scopel, A.L., Hutchings, M.J., 2013. The dynamics of three shrub species in a fire-prone temperate savanna: The interplay between the seed bank, seed rain and fire regime. *Plant Ecol.* 214, 75–86. <https://doi.org/10.1007/s11258-012-0147-9>
- Gasparino, D., Malavasi, U.C., Malavasi, M. de M., Souza, I. de, 2006. Quantificação do banco de sementes sob diferentes usos do solo em área de domínio ciliar. *Rev. Árvore* 30, 1–9. <https://doi.org/10.1590/s0100-67622006000100001>
- Gonnet, J.M., 2001. Influence of cattle grazing on population density and species richness of granivorous birds (Emberizidae) in the arid plain of the Monte, Argentina. *J. Arid Environ.* 48, 569–579. <https://doi.org/10.1006/jare.2000.0777>

- Grime, J.P., 1979. *Plant strategies and vegetation process*, 1 st. ed. John Wiley and Sons, Chichester.
- Han, J., Chen, J., Xia, J., Li, L., 2015. Grazing and watering alter plant phenological processes in a desert steppe community. *Plant Ecol.* 216, 599–613.  
<https://doi.org/10.1007/s11258-015-0462-z>
- Haretche, F., Rodríguez, C., 2006. Banco de semillas de un pastizal uruguayo bajo diferentes condiciones de pastoreo. *Ecol. Austral* 16, 105–113.
- Janzen, D.H., 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *Am. Nat.*  
<https://doi.org/10.1086/284208>
- Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L., Hölzel, N., 2010. Species introduction in restoration projects - Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic Appl. Ecol.* 11, 285–299.  
<https://doi.org/10.1016/j.baae.2009.12.004>
- Kiehl, K., Wagner, C., 2006. Effect of Hay Transfer on Long-Term Establishment of Vegetation and Grasshoppers on Former Arable Fields. *Restor. Ecol.* 14, 157–166.  
<https://doi.org/10.1111/j.1526-100X.2006.00116.x>
- Kinucan, R.J., Smeins, F.E., 1992. Soil Seed Bank of a Semiarid Texas Grassland Under Three Long-Term (36- Years) Grazing Regimes. *Am. Midl. Nat.* 128, 11–21.  
<https://doi.org/10.2307/2426408>
- Kolb, R.M., Pilon, N.A.L., Durigan, G., 2016. Factors influencing seed germination in Cerrado grasses . *Acta Bot. Bras.* .
- Kollmann, J., Goetze, D., 1998. Notes on seed traps in terrestrial plant communities. *Flora* 193, 31–40. [https://doi.org/10.1016/S0367-2530\(17\)30813-7](https://doi.org/10.1016/S0367-2530(17)30813-7)
- Le Stradic, S., Buisson, E., Fernandes, G.W., 2014. Restoration of Neotropical grasslands degraded by quarrying using hay transfer. *Appl. Veg. Sci.* 17, 482–492.  
<https://doi.org/10.1111/avsc.12074>
- Le Stradic, S., Buisson, E., Fernandes, G.W., Morellato, L.P.C., 2018. Reproductive phenology of two co-occurring Neotropical mountain grasslands. *J. Veg. Sci.* 29, 15–24.  
<https://doi.org/10.1111/jvs.12596>
- Lemon, A.J., Bolker, B., Oom, S., Klein, E., Rowlingson, B., Wickham, H., Tyagi, A., Eterradosi, O., Grothendieck, G., Toews, M., Kane, J., Turner, R., Witthoft, C., Stander, J., Petzoldt, T., Duursma, R., Biancotto, E., Levy, O., Dutang, C., Solymos, P., Engelmann, R., Hecker, M., Steinbeck, F., Borchers, H., Singmann, H., Toal, T., Ogle, D., Lemon, M.J., 2016. Package ‘plotrix.’
- Lennartsson, T., Wissman, J., Bergström, H.M., 2012. The effect of timing of grassland management on plant reproduction. *Int. J. Ecol.* 2012.  
<https://doi.org/10.1155/2012/156274>
- Leon, R.J.C., Bertiller, M., 1982. Aspectos fenológicos de dos comunidades del pastizal de la Depresion del Salado. *Bol. la Soc. Argentina Bot.* 20, 329–348.
- Lezama, F., Baeza, S., Altesor, A., Cesa, A., Chaneton, E.J., Paruelo, J.M., 2014. Variation of grazing-induced vegetation changes across a large-scale productivity gradient. *J. Veg. Sci.* <https://doi.org/10.1111/jvs.12053>
- Li, Yu, Dong, S., Gao, Q., Zhang, Y., Liu, S., Swift, D., Ganjurjav, H., Hu, G., Wang, X., Yan, Y., Wu, H., Luo, W., Ge, Y., Li, Yan, Zhao, Z., Gao, X., Li, S., Song, J., 2019. The effects of grazing regimes on phenological stages, intervals and divergences of alpine

- plants on the Qinghai-Tibetan Plateau. *J. Veg. Sci.* 30, 134–145.  
<https://doi.org/10.1111/jvs.12703>
- Linabury, M.C., Turley, N.E., Brudvig, L.A., 2019. Insects remove more seeds than mammals in first-year prairie restorations. *Restor. Ecol.* <https://doi.org/10.1111/rec.13004>
- Londo, G., 1976. The decimal scale for relevés of permanent quadrats. *Vegetatio.*  
<https://doi.org/10.1007/BF00055300>
- Loydi, A., Zalba, S.M., Distel, R.A., 2012. Viable seed banks under grazing and exclosure conditions in montane mesic grasslands of Argentina. *Acta Oecologica* 43, 8–15.  
<https://doi.org/10.1016/j.actao.2012.05.002>
- Lucero, J.E., Callaway, R.M., 2018. Granivory from native rodents and competition from an exotic invader strongly and equally limit the establishment of native grasses. *Oecologia.*  
<https://doi.org/10.1007/s00442-018-4085-7>
- Maarel, E., Sykes, M.T., 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *J. Veg. Sci.* 4, 179–188.  
<https://doi.org/10.2307/3236103>
- Marone, L., Rossi, B.E., De Casenave, J.L., 1998. Granivore impact on soil-seed reserves in the central Monte desert, Argentina. *Funct. Ecol.* 12, 640–645.  
<https://doi.org/10.1046/j.1365-2435.1998.00226.x>
- Márquez, S., Funes, G., Cabido, M., Pucheta, E., Multidisciplinario, I., Vegetal, D.B., Cátedra, I., Geofísica, D. De, Biología, A., Exactas, F.D.C., Naturales, F., Nacional, U., Juan, D.S., Juan-argentina, S., 2002. Efectos del pastoreo sobre el banco de semillas germinable y la vegetación establecida en pastizales de montaña del centro de Argentina  
 Grazing effects on the germinable seed bank and standing vegetation in mountain grasslands from central Argentina *El pa* 327–337.
- Marteinsdóttir, B., 2014. Seed rain and seed bank reveal that seed limitation strongly influences plant community assembly in grasslands. *PLoS One* 9, e103352.  
<https://doi.org/10.1371/journal.pone.0103352>
- Martínková, J., Smilauer, P., Mihalka, S., 2002. Phenological pattern of grassland species: relation to the ecological and morphological traits. *Flora - Morphol. Distrib. Funct. Ecol. Plants* 197, 290–302. <https://doi.org/https://doi.org/10.1078/0367-2530-00043>
- Milchunas, D.G., Sala, O.E., Lauenroth, W.K., 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* 132, 87–106.  
<https://doi.org/10.1086/284839>
- Minervini-Silva, G.H., Overbeck, G.E., 2020. Soil seed bank in a subtropical grassland under different grazing intensities. *Acta Bot. Brasilica* 34, 360–370.  
<https://doi.org/10.1590/0102-33062019abb0297>
- Minervini Silva, G.H., Overbeck, G.E., 2021. Seasonal patterns of endozoochory by cattle in subtropical grassland in southern Brazil. *Austral Ecol. aec.*13054.  
<https://doi.org/10.1111/aec.13054>
- Morellato, L.P.C., Alberti, L.F., Hudson, I.L., 2010. Applications of circular statistics in plant phenology: A case studies approach, in: *Phenological Research: Methods for Environmental and Climate Change Analysis.* Springer Netherlands, pp. 339–359.  
[https://doi.org/10.1007/978-90-481-3335-2\\_16](https://doi.org/10.1007/978-90-481-3335-2_16)
- Müller, J., Heinze, J., Joshi, J., Boch, S., Klaus, V.H., Fischer, M., Prati, D., 2014. Influence of experimental soil disturbances on the diversity of plants in agricultural grasslands. *J.*

- Plant Ecol. 7, 509–517. <https://doi.org/10.1093/jpe/rtt062>
- Nabinger, C., Ferreira, E.T., Freitas, A.K., Carvalho, P.C.F., Sant’Anna, D.M., 2009. Produção animal com base no campo nativo: aplicações de resultados de pesquisa., in: Campos Sulinos: Conservação e Uso Sustentável Da Biodiversidade.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package. R package version 2.4-2. Community Ecol. Packag.
- Oleques, S.S., Overbeck, G.E., de Avia, R.S., 2017. Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. *Flora Morphol. Distrib. Funct. Ecol. Plants* 229, 141–146. <https://doi.org/10.1016/j.flora.2017.02.024>
- Oleques, S.S., Vizenin-Bugoni, J., Overbeck, G.E., 2019. Influence of grazing intensity on patterns and structuring processes in plant–pollinator networks in a subtropical grassland. *Arthropod. Plant. Interact.* 13, 757–770. <https://doi.org/10.1007/s11829-019-09699-8>
- Overbeck, G.E., Hermann, J.M., Andrade, B.O., Boldrini, I.I., Kiehl, K., Kirmer, A., Koch, C., Kollmann, J., Meyer, S.T., Müller, S.C., Nabinger, C., Pilger, G.E., Trindade, J.P.P., Vélez-Martin, E., Walker, E.A., Zimmermann, D.G., Pillar, V.D., 2013. Restoration ecology in Brazil-time to step out of the forest. *Nat. a Conserv.* 11, 92–95. <https://doi.org/10.4322/natcon.2013.015>
- Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C., Boldrini, I.I., Both, R., Forneck, E., 2007. Brazil’s neglected biome: The South Brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* 9, 101–116. <https://doi.org/10.1016/j.ppees.2007.07.005>
- Overbeck, G.E., Müller, S.C., Pillar, V.D., Pfadenhauer, J., 2005. Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *J. Veg. Sci.* 16, 655–664. <https://doi.org/10.1111/j.1654-1103.2005.tb02408.x>
- Palma, A.C., Laurance, S.G.W., 2015. A review of the use of direct seeding and seedling plantings in restoration: What do we know and where should we go? *Appl. Veg. Sci.* 18, 561–568. <https://doi.org/10.1111/avsc.12173>
- Pasini, E., Katinas, L., Ritter, M.R., 2014. O gênero *Chaptalia* ( Asteraceae , Mutisieae ) no Rio Grande do Sul , Brasil. *Rodriguésia* 65, 141–158.
- Peters, D.P.C., 2000. Climatic variation and simulated patterns in seedling establishment of two dominant grasses at a semi-arid-arid grassland ecotone. *J. Veg. Sci.* <https://doi.org/10.2307/3246579>
- Pillar, V.D.P., 2006. User s Guide v. 2.4. Memory.
- Pinheiro, M., Abrão, B.E. de, Harter-Marques, B., Miotto, S.T.S., 2008. Floral resources used by insects in a grassland community in Southern Brazil. *Rev. Bras. Botânica* 31. <https://doi.org/10.1590/s0100-84042008000300011>
- R Core Team (2020), 2020. R: A language and environment for statistical computing. R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. Vienna, Austria.
- R Core Team 2019, 2019. : A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL <http://www.R-project.org/>.
- Roberts, H.A., 1981. Seed banks in soils. *Adv. Appl. Biol.*
- Sale, P.F., 1977. Maintenance of High Diversity in Coral Reef Fish Communities. *Am. Nat.*

- 111, 337–359. <https://doi.org/10.1086/283164>
- Shmida, A., Ellner, S., 1984. Coexistence of plant species with similar niches. *Vegetatio* 58, 29–55. <https://doi.org/10.1007/BF00044894>
- Silva, I.A., Guido, A., Müller, S.C., 2020. Predicting plant performance for the ecological restoration of grasslands: the role of regenerative traits. *Restor. Ecol.* <https://doi.org/10.1111/rec.13182>
- Stucky, B.J., Guralnick, R., Deck, J., Denny, E.G., Bolmgren, K., Walls, R., 2018. The plant phenology ontology: A new informatics resource for large-scale integration of plant phenology data. *Front. Plant Sci.* 9, 1–12. <https://doi.org/10.3389/fpls.2018.00517>
- Thomas, P., Overbeck, G., Müller, S., 2019. Restoration of abandoned subtropical highland grasslands in Brazil: mowing produces fast effects, but hay transfer does not, *Acta Botanica Brasilica*. <https://doi.org/10.1590/0102-33062018abb0377>
- Thompson, K., Bakker, J.P., Bekker, R.M., 1997. Seed bank classification, in: *The Soil Seed Banks of North West Europe: Methodology, Density and Longevity*. Cambridge University Press, Melbourne, p. 276.
- Török, P., Migléc, T., Valkó, O., Kelemen, A., Tóth, K., Lengyel, S., Tóthmérész, B., 2012. Fast restoration of grassland vegetation by a combination of seed mixture sowing and low-diversity hay transfer. *Ecol. Eng.* 44, 133–138. <https://doi.org/10.1016/j.ecoleng.2012.03.010>
- Veldman, J.W., Buisson, E., Durigan, G., Fernandes, G.W., Le Stradic, S., Mahy, G., Negreiros, D., Overbeck, G.E., Veldman, R.G., Zaloumis, N.P., Putz, F.E., Bond, W.J., 2015. Toward an old-growth concept for grasslands, savannas, and woodlands. *Front. Ecol. Environ.* 13, 154–162. <https://doi.org/10.1890/140270>
- Vieira, M. de S., Bonilha, C.L., Boldrini, I.I., Overbeck, G.E., 2015. The seed bank of subtropical grasslands with contrasting land-use history in southern Brazil. *Acta Bot. Brasilica* 29, 543–552. <https://doi.org/10.1590/0102-33062015abb0026>
- Vogel Ely, C., 2019. Sistemática e conservação das espécies sul-americanas não andinas de *Hypericum* L. (HYPERICACEAE). *Fed. Univ. Rio Gd. do Sul. Federal University of Rio Grande do Sul*.
- von Blanckenhagen, B., Poschlod, P., 2005. Restoration of calcareous grasslands: The role of the soil seed bank and seed dispersal for recolonisation processes. *Biotechnol. Agron. Soc. Environ. BASE* 9, 143–149.
- Wagner, M., Hulmes, S., Hulmes, L., Redhead, J.W., Nowakowski, M., Pywell, R.F., 2020. Green hay transfer for grassland restoration: species capture and establishment. *Restor. Ecol.* 1–11. <https://doi.org/10.1111/rec.13259>
- Wilson, J.B., Peet, R.K., Dengler, J., Pärtel, M., 2012. Plant species richness: The world records. *J. Veg. Sci.* 23, 796–802. <https://doi.org/10.1111/j.1654-1103.2012.01400.x>
- Zar, J., 2010. *Biostatistical analysis*. 5th, Pearson Prentice-Hall, Upper Saddle River.
- Zarco, A., Cueto, V.R., Sagario, M.C., Marone, L., 2019. Effects of livestock grazing on flocks of seed-eating birds in the central monte desert, Argentina. *Can. J. Zool.* <https://doi.org/10.1139/cjz-2018-0223>
- Zhu, J., Zhang, Y., Liu, Y., 2016. Effects of short-term grazing exclusion on plant phenology and reproductive succession in a Tibetan alpine meadow. *Sci. Rep.* 6, 2–10. <https://doi.org/10.1038/srep27781>



Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. Analyzing Ecological Data, Methods.  
<https://doi.org/10.1016/B978-0-12-387667-6.00013-0>

## Supplementary material

**Table 1.** Phenogram of the species found in the vegetation survey in subtropical grassland in southern Brazil during the twelve months of study (combined data across grazing treatments). Gray = phenophase 1 and 2 ('flowering'); black = phenophase 3 and 4 ('fruiting'). Numbers indicate how reproductive structures were quantified: individual flowers (1), inflorescence (2) or inflorescence branches (3).

Family	Species		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Set	Oct	Nov	Dec
Acanthaceae	<i>Justicia axillaris</i> (Nees) Lindau	1	Gray	Black	Gray							Gray	Black	Black
Acanthaceae	<i>Ruellia hypericoides</i> (Nees) Lindau	1		Gray	Black	Black	Black					Gray	Black	Black
Acanthaceae	<i>Ruellia morongii</i> Britton	1	Black	Gray	Black	Black	Black		Gray			Gray	Black	Black
Amaranthaceae	<i>Pfaffia tuberosa</i> (Spreng.) Hicken	2	Black	Black	Black	Black	Black					Black	Black	Black
Amaryllidaceae	<i>Nothoscordum bivalve</i> (L.) Britton	1										Black	Black	Black
Amaryllidaceae	<i>Nothoscordum gracile</i> (Aiton) Stearn	1									Gray	Black	Black	Black
Amaryllidaceae	<i>Nothoscordum montevidense</i> Beauverd	1									Gray	Black	Black	Black
Amaryllidaceae	<i>Zephyranthes americana</i> (Hoffmanns.) Ravenna	1		Black										
Anacardiaceae	<i>Schinus terebinthifolius</i> Raddi	2		Gray										
Apiaceae	<i>Centella asiatica</i> (L.) Urb.	2	Gray	Black	Black	Black	Black	Black	Gray			Black	Black	Black
Apiaceae	<i>Cyclospermum leptophyllum</i> (Pers.) Sprague	2									Gray	Black	Black	Black
Apiaceae	<i>Eryngium ciliatum</i> Cham. & Schltdl.	3	Black	Black								Black	Black	Black
Apiaceae	<i>Eryngium horridum</i> Malme	3	Black	Black	Black	Black	Black	Black	Black	Black			Black	Black
Araliaceae	<i>Hydrocotyle exigua</i> Malme	1				Gray			Gray			Gray		
Aristolochiaceae	<i>Aristolochia sessilifolia</i> (Klotzsch) Duch.	1	Black									Gray	Black	Black
Asteraceae	<i>Aspilia montevidensis</i> (Spreng.) Kuntze	2	Gray	Black	Black	Black	Black	Black	Black			Gray	Black	Black

Asteraceae	<i>Austroeupatorium inulaefolium</i> (Kunth) R.M.King & H.Rob.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Baccharis anomala</i> DC.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Baccharis articulata</i> (Lam.) Pers.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Baccharis coridifolia</i> DC.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Baccharis crispa</i> Spreng.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Baccharis dracunculifolia</i> DC.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Baccharis riograndensis</i> Malag. & J.Vidal	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Bidens pilosa</i> Linn	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chaptalia exscapa</i> (Pers.) Baker	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chaptalia integerrima</i> (Vell.) Burkart	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chaptalia piloselloides</i> (Vahl) Baker	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chaptalia runcinata</i> Kunth	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chevreulia acuminata</i> Less.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chevreulia sarmentosa</i> (Pers.) S.F.Blake	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chromolaena congesta</i> (Hook. & Arn.) R.M.King & H.Rob.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chromolaena laevigata</i> (Lam.) R.M.King & H.Rob.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Chrysolaena flexuosa</i> (Sims) H.Rob.	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Conyza blakei</i> (Cabrera) Cabrera	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Conyza bonariensis</i> (L.) Cronquist	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Conyza primulifolia</i> (Lam.) Cuatrec. & Lourteig	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█
Asteraceae	<i>Elephantopus mollis</i> Kunth	2	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█









Oxalidaceae	<i>Oxalis lasiopetala</i> Zucc.	1							
Oxalidaceae	<i>Oxalis lindneri</i> Knuth	1							
Plantaginaceae	<i>Cyclospermum leptophyllum</i> (Pers.) Sprague ex Britton & P.Wilson	2							
Plantaginaceae	<i>Mecardonia procumbens</i> var. <i>tenella</i> (Cham. & Schtdl.) V.C.Souza	1							
Plantaginaceae	<i>Plantago australis</i> Lam.	2							
Plantaginaceae	<i>Plantago myosurus</i> Lam.	2							
Plantaginaceae	<i>Plantago penantha</i> Griseb.	2							
Plantaginaceae	<i>Plantago tomentosa</i> Lam.	2							
Plantaginaceae	<i>Veronica serpyllifolia</i> L.	1							
Poaceae C3	<i>Chascolytrum lamareckianum</i> (Nees) Matthei	3							
Poaceae C3	<i>Chascolytrum poomorphum</i> (J. Presl) L. Essi, Longhi-Wagner & Souza-Chies	3							
Poaceae C3	<i>Chascolytrum rufum</i> J. Presl	3							
Poaceae C3	<i>Chascolytrum subaristatum</i> (Lam.) Desv.	3							
Poaceae C3	<i>Chascolytrum uniolae</i> (Nees) L. Essi, Longhi-Wagner & Souza-Chies	3							
Poaceae C3	<i>Melica brasiliana</i> Ard.	3							
Poaceae C3	<i>Nassella neesiana</i> (Trin. & Rupr.) Barkworth	3							
Poaceae C3	<i>Nassella nutans</i> (Hack.) Barkworth	3							
Poaceae C3	<i>Piptochaetium montevidense</i> (Spreng.) Parodi	3							
Poaceae C3	<i>Piptochaetium ruprechtianum</i> E.Desv.	3							
Poaceae C3	<i>Piptochaetium stipoides</i> (Trin. & Rupr.) Hack. & Arechav.	3							
Poaceae C3	<i>Steinchisma hians</i> (Elliott) Nash	3							
Poaceae C4	<i>Andropogon bicornis</i> L.	3							



Poaceae C4	<i>Andropogon lateralis</i> Nees	3	
Poaceae C4	<i>Andropogon selloanus</i> (Hack.) Hack.	3	
Poaceae C4	<i>Andropogon ternatus</i> (Spreng.) Nees	3	
Poaceae C4	<i>Aristida filifolia</i> (Arechav.) Herter	3	
Poaceae C4	<i>Aristida jubata</i> (Arechav.) Herter	3	
Poaceae C4	<i>Aristida laevis</i> (Nees) Kunth	3	
Poaceae C4	<i>Aristida venustula</i> Arechav.	3	
Poaceae C4	<i>Axonopus affinis</i> Chase	3	
Poaceae C4	<i>Axonopus fissifolius</i> (Raddi) Kuhlmann	3	
Poaceae C4	<i>Dichantherium sabulorum</i> (Lam.) Gould & C.A.Clark	3	
Poaceae C4	<i>Digitaria violascens</i> Link	3	
Poaceae C4	<i>Elionurus candidus</i> (Trin.) Hack.	3	
Poaceae C4	<i>Eragrostis airoides</i> Nees	3	
Poaceae C4	<i>Eragrostis bahiensis</i> Schrad. ex Schult.	3	
Poaceae C4	<i>Eragrostis lugens</i> Nees	3	
Poaceae C4	<i>Eragrostis neesii</i> Trin.	3	
Poaceae C4	<i>Eragrostis polytricha</i> Nees	3	
Poaceae C4	<i>Microchloa indica</i> (L.f.) P. Beauv.	3	
Poaceae C4	<i>Mnesithea selloana</i> (Hack.) de Koning & Sosef	3	
Poaceae C4	<i>Panicum beergi</i> Arechav.	3	
Poaceae C4	<i>Panicum prionitis</i> Nees	3	

Poaceae C4	<i>Paspalum lepton</i> Schult.	3	
Poaceae C4	<i>Paspalum notatum</i> Flügge	3	
Poaceae C4	<i>Paspalum paniculatum</i> L.	3	
Poaceae C4	<i>Paspalum pauciciliatum</i> (Parodi) Herter	3	
Poaceae C4	<i>Paspalum paucifolium</i> Swallen	3	
Poaceae C4	<i>Paspalum plicatulum</i> Michx.	3	
Poaceae C4	<i>Paspalum pumilum</i> Nees	3	
Poaceae C4	<i>Saccharum angustifolium</i> (Nees) Trin.	3	
Poaceae C4	<i>Sacciolepis vilvoides</i> Chase	3	
Poaceae C4	<i>Schizachyrium condensatum</i> (Kunth) Nees	3	
Poaceae C4	<i>Schizachyrium spicatum</i> (Spreng.) Herter	3	
Poaceae C4	<i>Schizachyrium tenerum</i> Nees	3	
Poaceae C4	<i>Setaria parviflora</i> (Poir.) M. Kerguelen	3	
Poaceae C4	<i>Setaria vaginata</i> Spreng.	3	
Poaceae C4	<i>Sorghastrum setosum</i> (Griseb.) Hitchc.	3	
Poaceae C4	<i>Sporobolus indicus</i> (L.) R.Br.	3	
Polygalaceae	<i>Polygala aphylla</i> A.W.Benn.	2	
Polygalaceae	<i>Polygala australis</i> A.W. Benn.	2	
Polygalaceae	<i>Polygala linooides</i> Poir.	2	
Polygalaceae	<i>Polygala pulchella</i> A.St.-Hil. & Moq.	2	



**CAPÍTULO 3: Seedling emergence from seed rain in subtropical grasslands under different grazing intensities<sup>3</sup>**

Graziela Har Minervini Silva<sup>123</sup>, Luciana da Silva Menezes<sup>1</sup>, Gerhard Ernst Overbeck<sup>12</sup>

<sup>1</sup>Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Botânica. Avenida Bento Gonçalves 9500, Porto Alegre, RS, Brasil.

<sup>2</sup>Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Botânica. Avenida Bento Gonçalves 9500, Porto Alegre, RS, Brasil.

<sup>3</sup>Corresponding address: graziela.minervini@gmail.com

---

<sup>3</sup> Este manuscrito será submetido à revista *Rangeland Ecology & Management*.

## **Abstract**

Seed rain is essential for the maintenance of propagule stocks in the seed bank and for plant recruitment and thus plant community assembly, but seed rain studies in Southern Hemisphere grasslands are still rare. Further, very little is known on the effects of grazing, a common type of land management, on dispersal of diaspores by seed rain. We evaluated the seed rain in grasslands with distinct grazing intensities (high, moderate, low and very low) and an ungrazed grassland in subtropical southern Brazil. Monthly, during one year. Seed rain was collected using pot traps with sterile soil. Additionally, fruiting species in the vegetation were recorded. The seed rain was estimated by the seedling emergence method. We performed randomization tests to compare seed rain density and composition among managements and seasons and evaluated functional composition of the seed rain (broad functional groups of plants and dispersal traits). We observed high number of seedlings in seed rain. Summer samples and the ungrazed treatment showed the highest number of seedlings. Similarity between seed rain and vegetation was low (Sørensen index values between 13 - 30%). The functional groups composition varied between treatments and seasons, indicating that the peaks of dispersal are related to grazing and to changes in environmental conditions over the year that act on the plant physiology, determining the phenophases. Pot traps appeared to be suitable for analysis of seed rain.

**Keywords:** Propagules, fruiting, anemochory, autochory, colonization, seedling emergence, seed availability, management, grazing intensities.

## **Introduction**

Seed rain, the cumulatively new produced seeds that can enter into the soil seed bank (SSB) after production (Marone et al., 1998) is an important process in plant community assembly (Marteinsdóttir, 2014). It is crucial for maintenance of propagule stocks in the seed bank and for the long-term persistence of populations of plants that rely on sexual reproduction (Galíndez et al., 2013). The presence of a reservoir of propagules in a plant community, either already present in the soil or dispersed by seed rain, largely depends on the landscape setting and land-use history of the studied ecosystems (Müller et al., 2014). Species with a persistent SSB or with the capacity of long-distance dispersal (e.g. by anemochory or zoochory) are more successful to re-establish their populations within short periods (Cosyns et al., 2005; von Blanckenhagen and Poschlod, 2005). However, the transient seed bank, composed by species present in the established vegetation and in the upper soil layer (Thompson et al., 1997), is prevalent in the SSB in some grassland communities (Funes et al., 2001; Márquez et al., 2002). Species with a transient SSB depend on the supply of seeds dispersed from adjacent areas (i.e., seed rain) for their regeneration when they disappear from the established vegetation. The availability of propagules and, to a lesser extent, the availability of adequate spaces for germination, are well known as the determining factors in plant community assembly (Marteinsdóttir, 2014; Müller et al., 2014). The understanding of seed dispersal processes thus is important to understand the dynamics of plant communities.

Most seed rain studies on grassland are concentrated in temperate regions and, in terms of geographical distribution, in Europe and North America (Arruda et al., 2018). In subtropical and tropical grassland in the Southern Hemisphere, seed rain studies are still rare (Arruda et al., 2018). Different studies on grassland dynamics in subtropical South America have demonstrated that grazing exerts strong influences on plant community composition and species abundance, both in established vegetation (Boavista et al., 2019; Cruz et al., 2010; Fischer et

al., 2019) and in the soil seed bank (Haretche and Rodríguez, 2006; Loydi et al., 2012; Vieira et al., 2015; Minervini-Silva and Overbeck, 2020). Grazing intensity also influences functional attributes of vegetation. For instance, in areas with high grazing intensity sites, prostrate grasses with a strategy of rapid resource acquisition dominate (Cruz et al. 2010), often with a high incidence of ruderal and/or non-perennial species. In contrast, sites with moderate grazing intensity are characterized vegetation clearly structure into a lower and an upper layer, with prostrate species in the first and caespitose species in the latter, sometimes with presence also of small shrubs. Sites with low grazing intensity are characterized by vegetation composed mainly of caespitose grasses and taller shrubs, with only small participation of prostrate species. (Adler et al., 2001; Nabinger et al., 2009 Cruz et al., 2010; Fedrigo et al., 2018). However, if, and to what magnitude, grazing affects seed production and dispersal processes, including if grazing intensity influences the number of viable diaspores in seed rain, is still largely unknown. This information is important to evaluate the effects of grazing management, including the potential of recovery after periods of overgrazing (Fedrigo et al., 2018).

Here we compared, over four seasons, the seed rain from grasslands under four grazing intensities and in a grassland without grazing (grazing exclusion) for over 10 years. We describe the seedling community that emerged from seed rain in terms of functional groups. We also compared the species composition from the seed rain with the species composition of the established vegetation. Our expectations were that (i) seed rain should be higher in summer, especially for C4 grasses and subshrubs, and in autumn, mainly for dicotyledonous herbs and species with pappus; (ii) seed rain will be mainly composed by species present in the established vegetation for low grazing intensity and grazing exclusion treatments, where the vegetation is taller (compared to heavily grazed areas) and acts as a barrier for seeds dispersed from adjacent communities (Bullock and Moy, 2004).

## Material and methods

### *Study site and experimental design*

The study was performed in an experimental area of the Agronomic Experimental Station of the Universidade Federal do Rio Grande do Sul (EEA-UFRGS) in Eldorado do Sul, Rio Grande do Sul state, Brazil (30°06'13"S and 51°40'55"W). Climate in the region is subtropical humid with hot summer and no dry season (Köppens' Cfa) (Alvares et al., 2013). The main vegetation type in the region is grassland with dominance of Poaceae (including C3 and C4 species), Asteraceae, Fabaceae and Cyperaceae in terms of species number (Andrade et al., 2019). During our study period (January to December 2016) the total precipitation was 1690 mm and temperatures varied from 5.9 °C to 31.8 °C (data provided by the weather station of the EEA-UFRGS) (Table 1).

**Table 1.** Average maximum and minimum monthly temperatures and monthly precipitation during the study period (January to December 2016). Data provided by the weather station of the Agronomic Experimental Station of UFRGS.

Seasons	Months	Maximum temperature (°C)	Minimum temperature (°C)	Precipitation (mm)
<i>Summer</i>	January	31.8	20.1	36
	February	31.6	20.4	189
	March	27.5	18.1	253
<i>Autumn</i>	April	26.6	17.9	305
	May	19.6	10.4	75
	June	15.9	5.9	11
<i>Winter</i>	July	19.3	8.4	164.5
	August	21.8	9.9	108
	September	21.5	10.8	77.5
<i>Spring</i>	October	24.2	14.2	253
	November	26.9	13.7	132
	December	30.3	18.8	86



Within EEA-UFRGS, a grassland management experiment with different levels of forage allowances (FA) and thus cattle grazing intensity (high, moderate, low and very low) is ongoing since 1986. The experimental treatments are daily forage allowances of 4, 8, 12, and 16 kg of dry plant biomass per 100 kg of cattle live weight. The 4% treatment represents high grazing intensity and the 16% treatment represents very low grazing intensity (for details: Cruz et al. 2010; Fischer et al. 2019). Plant biomass and animal weight are monitored monthly and stocking rates are adjusted accordingly to maintain the forage levels over the year. We used as control an adjacent ungrazed area which had not been grazed by cattle in more than ten years (grazing exclusion). The paddocks of different FA vary from 3 to 5 hectares, with two replicates per treatment. The adjacent ungrazed site had 23 hectares in total, but sampling was conducted in two subareas (delimited with GPS, since this area has no fences) of approximately 3 ha each. In total our sampling thus comprised 10 sampling sites, with 2 replicates of each grazing treatment and two for grazing exclusion site.

#### *Seed rain and seedling emergence*

Our seed traps were circular plastic pots, as proposed by Chabrierie & Alard (2005), with diameter of 10.5 cm and depth of 6 cm, with small holes in the bottom to prevent water accumulation. Each seed trap received a mixture of 50% sterile substrate (sterilized in autoclave) homogenized with 50% of vermiculite to improve moisture retention. Three seed traps were placed into the soil with pot surface at the soil level at each sampling site. Immediately after installation the seed traps were watered. In grazing treatments, the seed traps were protected inside cages to avoid trampling by cattle; we moved cages and seed traps every two months to prevent influence of vegetation growth inside exclusion cages.

Seed traps were left in the field and replaced by new pots with sterile substrate every month, and this process was repeated during twelve months (January to December 2016), thus

yielding a total of 360 seed traps over the course of the study. The seed traps brought from the field were placed in greenhouse (ambient temperature condition and regular watering) for monitoring of seedling emergence (Roberts, 1981). Control pots with sterile soil were distributed at random among the seed traps brought from the field to monitor possible contamination from the seed rain inside the greenhouse; no seedling emergence were observed in any of the control pots. Seedling emergence was observed for twelve months for all samples. Specimens that could not be identified at the seedling stage was transplanted into a separate container until the flowering stage, when the taxonomic identification was performed using specific literature.

#### *Vegetation surveys*

Plots used for vegetation sampling were randomly located, with a minimum distance between plots of at least 50 m and of at least 20 m from fences of adjacent grazing treatments. Vegetation surveys were conducted in five permanent plots of 1 m<sup>2</sup> in each sampling site on the same dates of seed collector's replacement. In each plot, we identified all plant species, cover percentage for each species was eye estimated based on the canopy vertical projection on the ground in relation to the total 1 m<sup>2</sup> area. We used Londo's decimal scale of vegetation cover (Londo, 1976). All species and families scientific names recorded in study were verified using the Flora do Brasil 2020 database (Flora do Brasil, 2020).

#### *Data analysis*

Number of seedlings per seed trap was converted into number of seedlings per m<sup>2</sup>. For this conversion, we used the equation  $S = \frac{1}{3*(Ac)} * ns$ , where S= seedlings/m<sup>2</sup>, Ac= area of soil sample ( $\pi*r^2$ ), multiplied by 3, as we had three seed traps per site, and ns= number of germinated seedlings per sampling site, resulting in two replicates for each grazing intensity

and enclosure treatments. Although samplings were realized monthly, we grouped seedlings and established vegetation data by seasons (Table 1) to better illustrate the observed patterns.

To describe the community present in seed trap samples, we categorized all species in relation to principal functional groups (C3 grasses, C4 grasses, non-grass monocots/monocotyledoneous herbs, dicots/dicotyledoneous herbs, subshrubs), life cycle (non-perennial, i.e. annual and/or biannual, and perennial, Burkart, 1969), and presence of specific dispersal structures. We selected dispersal structures commonly present in grasslands species: (i) awns are a filiform extension of the central vein of glumes and, especially, lemmas, common in Poaceae species (e.g. *Aristidaa* spp., *Nassela* spp., *Piptochaetium* spp.) (Boldrini et al., 2008); (ii) wings are a dilated/expanded structure that extends from the surface of fruits and/or seeds (e.g. *Eryngium horridum*, *Soliva sessilis*); (iii) trichomes are a hair-like epidermal projections on seeds/propagules (e.g. *Desmodium incanum*, *Andropogon ssp.*); (iv) pappus are a modified calyx, hairy, and persistent that is found in the proximal and/or distal portion of the fruits and seeds, common in Asteraceae species (Duarte et al., 2019). We performed randomization tests comparing groups of sampling units, using Euclidean distance (10000 iterations) to compare densities, life cycle, principal functional groups, seed structures of seed trap samples among the grazing intensities and enclosure treatments and seasons.

To compare species composition from established vegetation with seed rain we conducted two nonmetric multidimensional scaling analyses (NMDS). In the established vegetation NMDS we used species mean abundance (cover) in each sampling site (average of five plots) per season (average of three months). For the seed rain NMDS we used as species abundance total number of seedlings emerged of each species per square meter inside sampling sites (sum of the three seed traps) per season (average of three months). Bray-Curtis dissimilarity was used in both NMDS analyses. We tested for the effect of different grazing treatments and seasonality through PERMANOVA, also using Bray-Curtis dissimilarity.

However, tests of homogeneity of dispersion (Anderson and Walsh, 2013) showed significant dispersion effects for seasonality. Therefore, we presented and interpreted only the results of grazing treatments on species composition of established vegetation and seed rain. We calculated Sørensen index (Zuur et al., 2007) as a measurement of similarity of the seedling samples and composition of vegetation, comparing pairs of vegetation and seedling within the same grazing treatment and season.

Randomization tests were conducted in MULTIV software (Pillar, 2006). NMDS, PERMANOVA and tests of homogeneity of dispersion were made in R (R Core Team, 2020), using vegan package (Oksanen et al., 2019) with the following respective functions: metaMDS, adonis2 and permdisper.

## Results

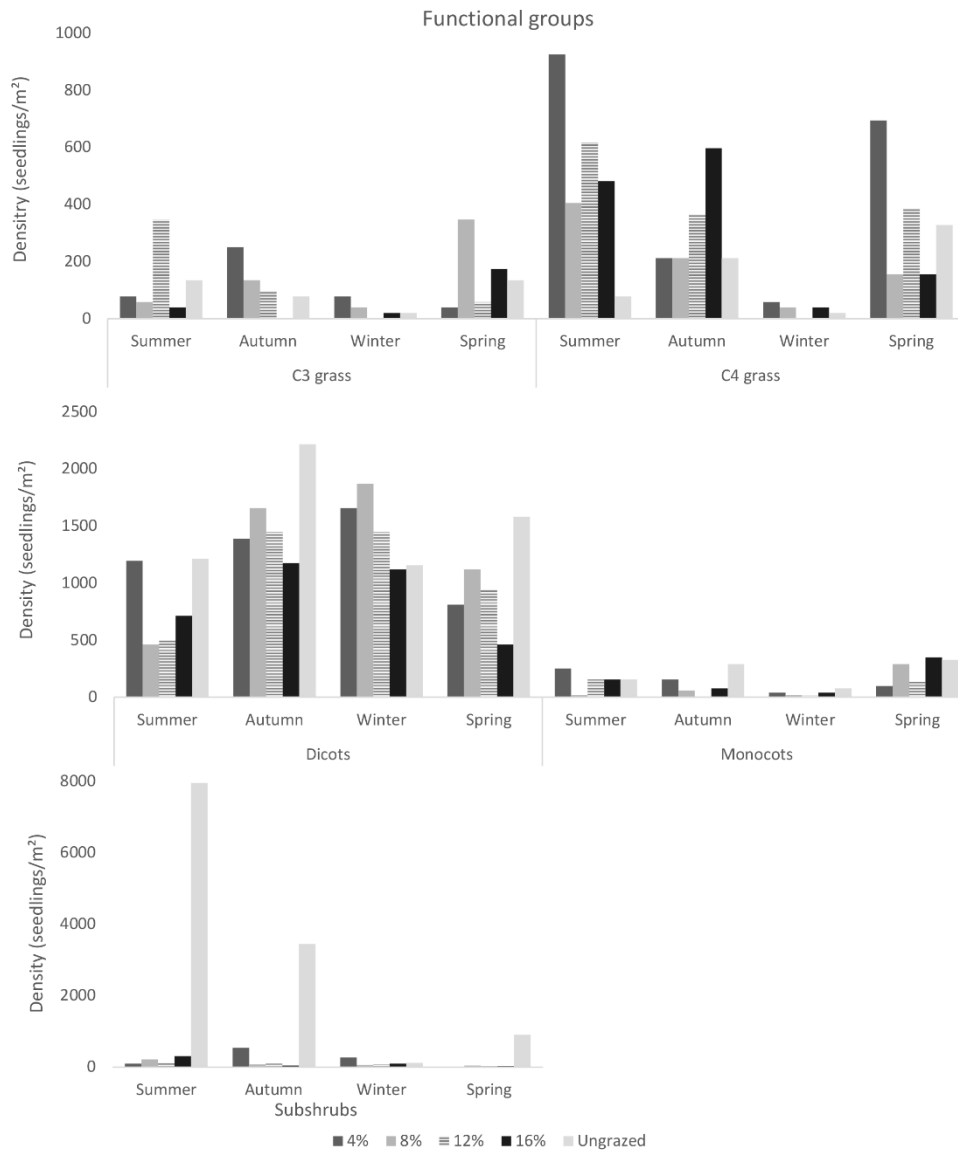
### *Seed trap content*

Among a total of 90 species captured in the seed traps, *Baccharis dracunculifolia* (19% of seedlings), *Cardamine chenopodiifolia* (16% of seedlings), *Gamochaeta coarctata* (9% of seedlings), *Hypericum caprifoliatum* and *Oxalis conorrhiza* (5% of seedlings for each), *Steinchisma hians* and *Axonopus affinis* (4% of seedlings for each) were the species with the highest density (Table S1). The 90 species were distributed in 22 families, of which Asteraceae (40% of seedlings) and Poaceae (16% of seedlings) were the most important in terms of total density. Summer samples had the highest number of seedlings, with a mean of 3,343 seedlings/m<sup>2</sup> (summing up the data from the three summer months), followed by autumn (2,961 seedlings/m<sup>2</sup>), spring (1,910 seedlings/m<sup>2</sup>) and winter with (1,672 seedlings/m<sup>2</sup>). The grazing exclusion treatment showed higher production of seedlings, compared to the other treatments, with 9,532 seedlings/m<sup>2</sup> in summer, 6,239 seedlings/m<sup>2</sup> in autumn and 3,274 seedlings/m<sup>2</sup> in spring (Table S1). The randomization tests showed that density of seedlings/m<sup>2</sup> varied between

autumn and winter (Table S2), and also between the ungrazed treatment and the other grazing intensity treatments (Table S2).

#### *Description of groups present in the seed traps*

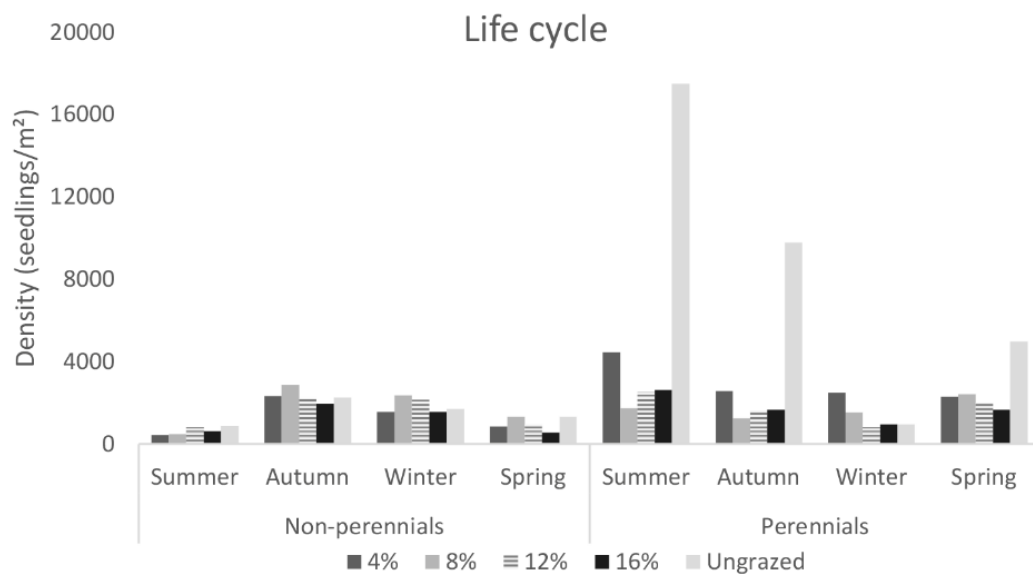
Regarding functional groups, 49% of the species were herbaceous dicots, 29% were subshrubs, 12% were C4 grasses, 6% were non-grass monocots, and 4% C3 grasses. Perennial plants corresponded to 69% of the species. Both C3 and C4 grasses had lower density in winter (Fig. 1) in comparison other seasons (Table S2). In summer and spring, C4 grasses showed high number of seedlings, especially at high grazing intensity (4% FA) (Fig. 1). We also observed that the C4 grasses intercalate the density peaks between low (12% FA) and very low (16% FA) grazing intensity in summer, autumn and spring (Fig. 1). Regarding management, no differences were found among treatments for C3 grasses (Table S2). C4 grasses presented highest number of seedlings/m<sup>2</sup> in high grazing intensity (4% FA) in relation the others treatments, with variation between moderate grazing intensity (8% FA) (Table S2) and grazing exclusion treatment (Table S2), where C4 grasses densities were lower. Dicot herbs showed higher density in autumn, with differences between spring and summer (Table S2), mainly in the moderate grazing intensity (8% FA) (Fig. 1), in contrast with lower density in very low grazing intensity (16% FA) (Table S2). Non-grass monocot density was higher in spring and summer than in autumn and winter (Table S2). Regarding management for non-grass monocot, grazing exclusion treatment differed from the other grazing intensities (Fig. 1; Table S2).



**Figure 1.** Density (seedlings/m<sup>2</sup>) of species in seed rain at the study site in Eldorado do Sul, RS, Brazil, according to functional groups, by season and grazing and ungrazed treatments. FA: forage allowance.

Non-perennial species showed prominence in density in autumn and winter, which presented differences with summer and spring (Table S2). We did not find significant differences in seedling density across among grazing treatments (Fig. 2, Table S2). Overall, perennial species showed higher density in relation to non-perennials. Perennial species showed higher density in the summer, and mainly in the grazing exclusion treatment (Fig. 2), which was significantly different from the other grazing intensity treatments (Table S2); except in winter, where the values were considerably lower compared to the other seasons, with gradually

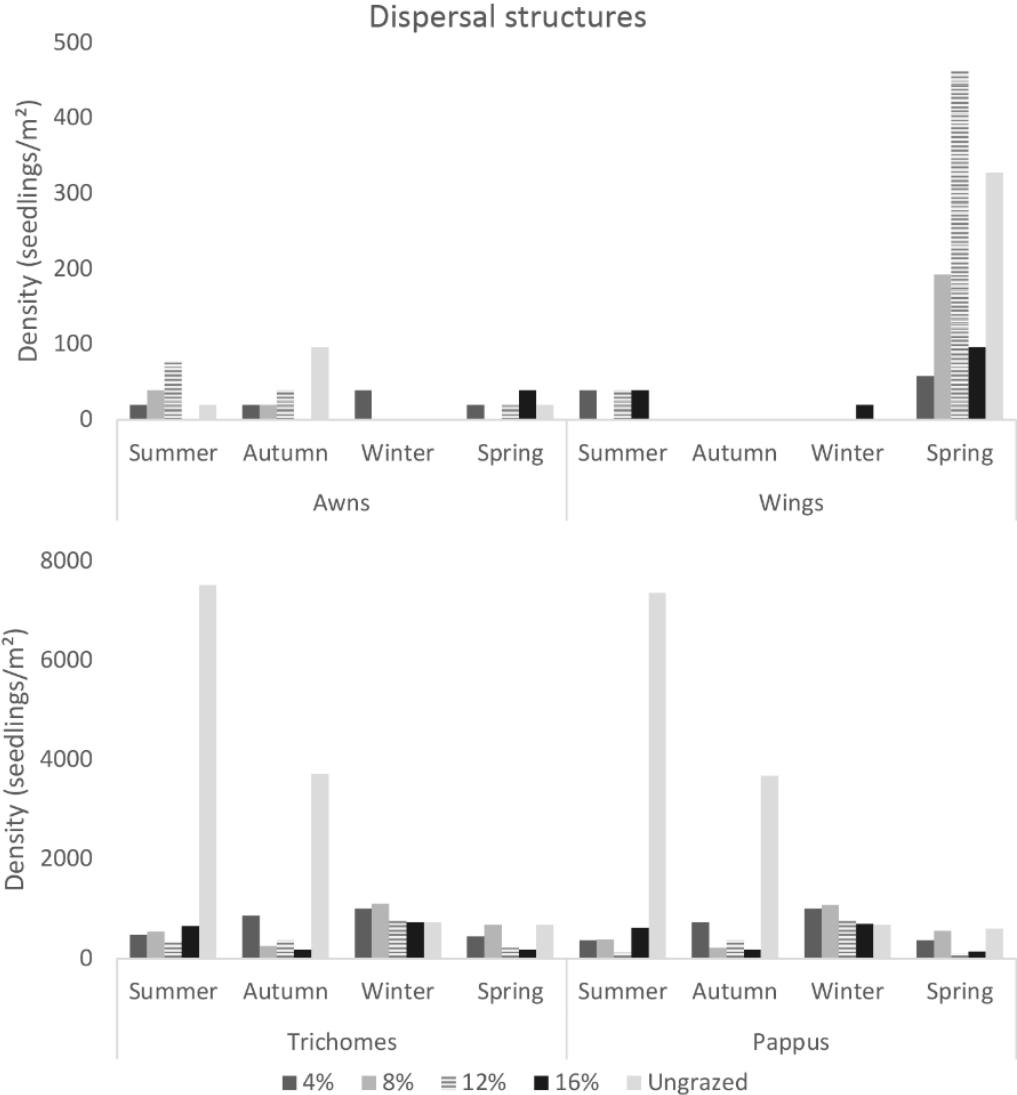
decreasing the high grazing intensity to the ungrazed treatment (Fig. 2). Randomization tests showed significant differences between summer and the other seasons and also between winter and spring (Table S2).



**Figure 2.** Density (seedlings/m<sup>2</sup>) of species in seed rain at the study site in Eldorado do Sul, RS, Brazil, according to life cycle, by season and grazing intensities and ungrazed treatments. FA: forage allowance.

Species with awns were most prominent in summer and autumn in treatments with low or no grazing, while in winter these species were recorded only in high and moderate grazing intensities (Fig. 3); however, awns did not show significant differences for seasons and for management treatments (Table S2). Species with wings were most abundant in spring, especially in very low grazing intensity (16% FA) and ungrazed treatments (Fig. 3). Randomization tests showed significant differences between summer and other seasons (Table S2), and spring between autumn and winter (Table S2) for wings; but no differences between managements were found. Species with trichomes and pappus were very abundant in relation to other dispersion structures, showing greater prominence in autumn and winter (Fig. 3). Significant differences were found between spring and winter for trichomes and pappus (Table S2), and also between spring and summer for trichomes (Table S2); regarding management,

both trichomes and pappus showed significant differences between the ungrazed treatment and the other grazing intensities (Table S2); also, differences were found between high (4% FA) and low (12% FA) grazing intensities for pappus (Table S2).



**Figure 3.** Density (seedlings/m<sup>2</sup>) of seed rain species regarding to dispersion structures, by season and grazing intensities and ungrazed treatments. FA: forage allowance.

*Relationship between seed traps records and established vegetation*

The established vegetation, sampled at 5 plots per experimental area, was composed by 197 species, from 35 families, of which 74 species were also found in the seed rain. Poaceae (57%), Asteraceae (16%), Apiaceae (12%) and Cyperaceae (6%) showed high contribution in



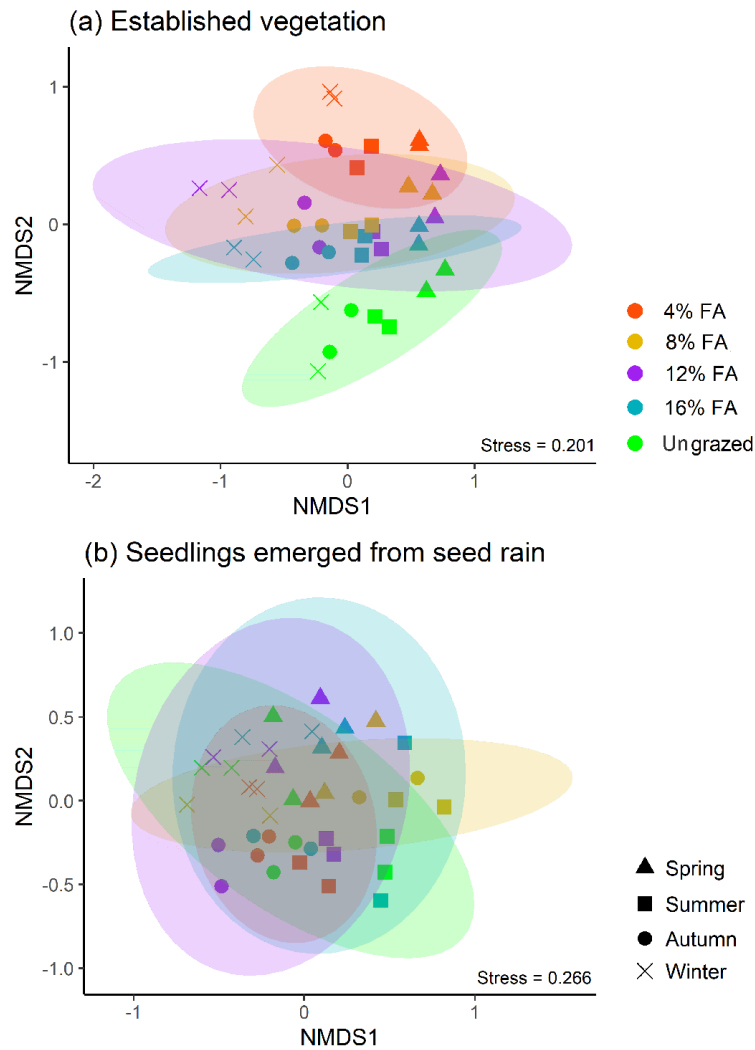
terms of vegetation cover in the sampled plots. *Eryngium horridum*, *Andropogon lateralis*, *Paspalum notatum*, *Baccharis crispa* e *Aristida laevis* were the species that had the highest percentages of vegetation cover (Table S3).

The similarities in the floristic composition between the samples of seed rain and the established vegetation were low, particularly in winter and at ungrazed sites (Table 2). Considering all management treatments, summer and spring presented greater similarity between seed rain and established vegetation, but no significant differences were found between seasons (Table 2). The moderate grazing intensity (8% FA) was the treatment that presented the highest similarity, considering the seasons indexes together (Table 2); the 8% FA treatment showed significant differences with the ungrazed treatment (Table S2), while the other treatments showed no differences.

**Table 2.** Sørensen index results (averages of the two replicates for each treatment) among species from the seed rain and the species present in the established vegetation at the study site in Eldorado do Sul, RS, Brazil. Data is given for grazing intensity/ungrazed treatments and season. FA: forage allowance.

	<b>4% FA</b>	<b>8% FA</b>	<b>12% FA</b>	<b>16% FA</b>	<b>Ungrazed</b>
<i>Summer</i>	26.51%	27.04%	25.02%	22.96%	21.92%
<i>Autumn</i>	16.54%	23.56%	26.87%	19.38%	15.01%
<i>Winter</i>	26.35%	24.44%	20.39%	13.25%	15.26%
<i>Spring</i>	27.41%	23.00%	23.14%	21.90%	20.83%

As observed in the NMDS analyses (Fig. 4), patterns of species composition were different between established vegetation and seed rain. Different grazing intensities were more responsible for driving changes in established vegetation ( $R^2= 0.37$ ,  $P= 0.001$ ) than seed rain ( $R^2= 0.15$ ,  $P= 0.001$ ).



**Figure 4.** Patterns of community composition from (a) established vegetation and (b) seedlings emerged from seed rain at the study site in Eldorado do Sul, RS, Brazil. Ellipses and colors indicate groups of different grazing intensity and ungrazed treatments, the effect was significant for established vegetation ( $R^2= 0.37$ ,  $P= 0.001$ ) and seed rain ( $R^2= 0.15$ ,  $P= 0.001$ ). Seasons are only indicated by different shapes, not tested. FA: forage allowance.

## Discussion

### *Seed rain in subtropical grasslands under distinct grazing intensities*

To the best of our knowledge, this is the first study to estimate seed rain in subtropical grasslands in southeastern South America. We focused on dispersal processes in grasslands with contrasting grazing intensities, including an ungrazed site, using pots exposed in the field as seed trap method. While other types of traps exist (e.g. funnel trap, that can catch a broad

range of seeds, and sticky trap that capture airborne seeds; see Chabrierie & Alard (2005), pot traps capture the seeds were actually deposited on or incorporated into the soil, and thus enable to identify which species present viable seeds that are ready to germinate under local conditions (Kollmann and Goetze, 1998). This means that pot trap data should give a realistic picture of the contribution of seed rain to the plant community. Another advantage of this type of trap is that incorporation of seeds into the soil acts as a possible protection against granivores. As traps were substituted monthly, we get a good indication of dispersal processes over the year. Our results provide primordial information on seed dispersal processes and applicability of the pot trap technique in the subtropical grasslands.

In calcareous grasslands in northwestern France, Chabrierie and Alard (2005) related very low efficiency in pot traps due to a low number of seeds (460 seeds/m<sup>2</sup>, 18 species) when compared to sticky traps (1,962 seeds/m<sup>2</sup> and 39 species) and funnel traps (8,079 seeds/m<sup>2</sup>, 56 species) in 12 months of study. Nonetheless, Chabrierie & Alard (2005) considered that in pot traps the smaller number of seeds captured is compensated by the fact that this kind of trap depicts the reality of the environmental filters acting at the study site. In our study, the method was much more effective; our results showed an overall mean of 2,471 seedlings/m<sup>2</sup> and 90 species for same trap and number of months studied. Obviously, comparisons between different grassland environments should be done with caution, since the effectiveness of the capture of seed rain may vary according to the vegetation at the study sites; clearly, we need more studies on the topic that should be conducted using standardized methods that will then allow us to compare different types of grassland (see also Arruda et al. 2018).

#### *Characterization of seed rain according to functional attributes*

The high density, in the seed rain, of perennial species at the ungrazed site is due to the high presence of Asteraceae shrubs, dominant in terms of canopy and seed investment with

pappus, such as *Baccharis dracunculifolia*, *Chromolaena congesta* and *Baccharis crispa*. The important role of seed rain for *B. dracunculifolia* populations has also been shown by Galíndez et al. (2013), especially for areas with lower population density due to higher disturbance level (in their case, fire). In contrast to this clear association of one species group, shrubs, to the ungrazed treatment, non-perennials, dicots and species with trichomes and pappus appeared in greater density across treatments. Many of the species with a combination of these characteristics share a ruderal character and have seeds that are easily dispersed by wind (Grime, 1979). Flowering and fruiting peaks of these species can vary considerably among species: peaks in spring and summer (*Gamochaeta americana*, *Chevreulia acuminata*), winter and spring (*G. calviceps*, *G. pennsylvanica*), July to February (*G. coarctata*) and throughout all the year (*Chaptalia pillosiloides*, *Chaptalia runcinata*) (Beretta et al., 2008; Deble and Marchiori, 2014; Pasini et al., 2014). C3 and C4 grasses showed variations among grazing intensity and ungrazed treatments in same season and also among seasons. Some species can advance phenophases to ensure their survival by sexual reproduction or delay flowering and fruiting to invest in the maintenance of vegetative parts to increase photosynthesis process (Lennartsson et al., 2012; Li et al., 2019). These variations during the seasons, and even the treatments, suggest that the peaks of flowering and fruiting activity may be more related to endogenous and abiotic factors (in our study those related to grazing and resource availability for species) that act on the physiology of the plant, determining or restricting the period of occurrence of phenophases (Benke and Morellato, 2002). Species with awns were abundant in terms of cover in established vegetation of low grazing intensity, but in our seed rain samples their density was low (e.g. *Andropogon lateralis* and *Schizachyrium microstachyum*) or null for some principal species (e.g. *Aristida* ssp. and *Nassella* ssp.). Possibly, species with larger awns (such as *Aristida* ssp. and *Nassella* ssp.) often do not manage to reach the ground, as they are caught by vegetation, even more so as they often stick together; for these species, possibly

larger patches of open soil are important for recruitment. The species with awns found in our pots present small awns and were found mainly in seed rain traps of low grazing intensity (12% FA) and ungrazed treatment. Despite the dominance of caespitose grasses and subshrubs, the low grazing intensity and ungrazed areas present more gaps of soil among the vegetation and less litter compared to the very low grazing intensity (16% FA), which can facilitate the arrival and incorporation of propagules in the soil. On the other hand, although the vegetation cover can act as a barrier for seed arrival, and thus capture, in the pot traps, the lower density of seedlings with awns may also be related to the low germination rates of these grasses. Silva et al. (2020) showed in an experiment that *Schizachyrium microstachyum*, *Aristida jubata* and *Aristida laevis* presented mean final germination percentage between 3.6 and 12.8% in the field, and 4.0 and 11.0% in the greenhouse, respectively.

#### *Similarity between seed rain and established vegetation*

We hypothesized that in treatments with a high forage allowance the similarity between the seed rain and the established vegetation would be greater, as taller vegetation should be acting as a barrier to the seed rain of the adjacent communities (Bullock and Moy, 2004). Thus, at very low grazing intensity (16% FA) and ungrazed sites, the seed rain should be composed predominantly by species from the local community. However, the values of the Sørensen's index were lower in these treatments. Possibly, in the very low grazing intensity the dominant caespitose grasses invest mainly in vegetative growth to better capture resources and light for photosynthesis, instead of investing in seeds (Lezama et al., 2014; Li et al., 2019). At ungrazed sites, *Baccharis dracunculifolia* shrubs were dominant. This species has a high production of propagules, but as under very low grazing intensity (16% FA), the high canopy cover monopolizes resources and impairs other less competitive species. We also observed that species such as *Cardamine chenopodifolia* and *Gamochaeta coarctata*, of a ruderal character,

due to the strong investment in seed production (Grime, 1979), presented a reasonable density in seed traps in these treatments with greater forage supply. The exclusion of grazing may have benefited the recruitment of *Hypericum caprifoliatum*, a species with high seed production and not tolerant to grazing, that was not found in the established vegetation and seed rain in the grazing sites, but with high density of seeds in the ungrazed site (Vogel Ely, 2019). The low similarity between the seed rain (and also the soil seed bank) and the established vegetation is generality reported in several studies in the tropical, subtropical and temperate grasslands in the southern hemisphere (Haretche and Rodríguez, 2006; Feldman et al., 2007; Vieira and Overbeck, 2020; Minervini-Silva and Overbeck, 2020). This is likely due the fact that the vegetation is mainly composed by perennial species that do not necessarily rely on seed rain for recruitment of new individuals for maintenance of their populations (Vieira et al., 2015). In high and moderate intensity (4% and 8% FA) the similarity between the seed rain and the established vegetation was higher, when compared to other treatments, may be related to the dominance of herbs, mostly ruderal ones, common in intensive grazing, and the lower height of the vegetation (Oleques et al., 2019) providing reduced barrier for dispersal.

### *Conclusions and perspectives*

The understanding of seed dispersal processes is of high relevance for grassland restoration and for grassland management, e.g. when the objective is to revert negative effects of overgrazing (e.g. Fedrigo et al., 2018). While pot traps were suitable for analysis of seed rain in grasslands with different grazing intensities and exclusion of grazing at community level, future studies should also apply the protocol proposed by Chabrerie and Alard (2005) to test whether there is also effectiveness in the capture of seed rain by the other two trap types (sticky and funnel traps). Arruda et al. (2018) discuss important issues for seed rain studies from a grassland restoration perspectives, mentioning, for instance, the need to critically evaluate and

report negative aspects of trap choices, to increment reference seed collections, and to study seed quality, availability, and dormancy-breaking mechanisms (Arruda et al., 2018). In addition, it might be helpful to create online databases with information on the propagules of each species (e.g. size, weight, structures adapted to dispersion, germination rate, ecological considerations of the species, photos, among others), information that is still lacking for grassland species in southeastern South America. Our results show that large numbers of seeds are dispersed and potentially incorporated into the soil seed bank in the grassland studied by us. A more detailed understanding of these processes, possibly focusing also on target species, has the potential to improve grassland management, for a conservation and restoration or production perspective.

### **Acknowledgements**

We thank members of the Laboratory for the Study of Grassland Vegetation (Levcamp), UFRGS, for assistance in field and greenhouse work and in plant identification, especially Lucas Pinheiro, Kassiane Helmicki, Helena Streit, Lukiel Oliveira, Mariana Vieira, Dióber Lucas, Bianca Andrade, Ingmar Staude, Juliana Schaefer, Pedro Silva Filho and Ilsi Boldrini. We also thank Miguel Dall’Agnol, UFRGS, for greenhouse space. This study was financed in part by the National Council for Scientific and Technological Development (CNPq) research grant to GEO (477618/2013-8) and in part by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001. GEO acknowledges CNPq productivity grant (310022/2015-0 and 310345/2018-9) and GHMS acknowledges CAPES scholarship.

### **References**

Adler, P., Raff, D., Lauenroth, W., 2001. The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia*. <https://doi.org/10.1007/s004420100737>

- Agostinelli, C., Lund, U., 2011. R package circular: Circular Statistics (version 0.4-3). CA Dep. Environ. Sci. Informatics Stat. Ca'Foscari Univ. Venice, Italy. UL Dep. Stat. Calif. Polytech. State Univ. San Luis Obispo, California, USA.
- Albert, Á.J., Mudrak, O., Jongepierova, I., Fajmon, K., Frei, I., ˇSevcikova, M., Klimešova, J., Doležal, J., 2019. Grassland restoration on ex-arable land by transfer of brush-harvested propagules and green hay. *Agric. Ecosyst. Environ.* 272, 74–82. <https://doi.org/10.1016/j.agee.2018.11.008>
- Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. *Meteorol. Zeitschrift* 22, 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecol. Monogr.* <https://doi.org/10.1890/12-2010.1>
- Andrade, B.O., Bonilha, C.L., Overbeck, G.E., Velez-Martin, E., Rolim, R.G., Bordignon, S.A.L., Schneider, A.A., Vogel Ely, C., Lucas, D.B., Garcia, E.N., dos Santos, E.D., Torchelsen, F.P., Vieira, M.S., Silva Filho, P.J.S., Ferreira, P.M.A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V.D., Boldrini, I.I., 2019. Classification of South Brazilian grasslands: Implications for conservation. *Appl. Veg. Sci.* 22, 168–184. <https://doi.org/10.1111/avsc.12413>
- Arruda, A.J., Buisson, E., Poschlod, P., Silveira, F.A.O., 2018. How have we studied seed rain in grasslands and what do we need to improve for better restoration? *Restor. Ecol.* <https://doi.org/10.1111/rec.12686>
- Baker, H.G., 1989. Some Aspects of the Natural History of Seed Banks, in: *Ecology of Soil Seed Banks*. pp. 9–21. <https://doi.org/10.1016/b978-0-12-440405-2.50007-5>
- Bazzaz, F.A., 1983. Characteristics of populations in relation to disturbance in natural and man-modified ecosystems., in: Mooney, H.A., Godron, M. (Eds.), *Disturbance and Ecosystems*. Berlin, pp. 259–275. [https://doi.org/10.1007/978-3-642-69137-9\\_17](https://doi.org/10.1007/978-3-642-69137-9_17)
- Benke, C.S.C., Morellato, L.P.C., 2002. Comparação de dois métodos de avaliação da fenologia de plantas, sua interpretação e representação. *Rev. Bras. Botânica* 25, 269–275. <https://doi.org/10.1590/s0100-84042002000300003>
- Beretta, M.E., Fernandes, A.C., Schneider, A.A., Ritter, M.R., 2008. A família Asteraceae no Parque Estadual de Itapua, Viamao, Rio Grande do Sul, Brasil. *Rev. Bras. Biociencia* 6, 189–216.
- Bergmeier, E., Matthas, U., 1996. Quantitative studies of phenology and early effects of non-grazing in Cretan phrygana vegetation. *J. Veg. Sci.* 7, 229–236. <https://doi.org/10.2307/3236323>
- Bischoff, A., Hoboy, S., Winter, N., Warthemann, G., 2018. Hay and seed transfer to re-establish rare grassland species and communities: How important are date and soil preparation? *Biol. Conserv.* 221, 182–189. <https://doi.org/https://doi.org/10.1016/j.biocon.2018.02.033>
- Boavista, L. da R., Trindade, J.P.P., Overbeck, G.E., Muller, S.C., 2019. Effects of grazing regimes on the temporal dynamics of grassland communities. *Appl. Veg. Sci.* 22, 326–335. <https://doi.org/10.1111/avsc.12432>
- Boldrini, I.I., Longhi-Wagner, H.M., Boechat, S. de C., 2008. *Morfologia e taxonomia de gramineas Sul-Rio-Grandenses*, 2 nd. ed. UFRGS Editora, Porto Alegre.



- Bond, W.J., Keeley, J.E., 2005. Fire as a global “herbivore”: The ecology and evolution of flammable ecosystems. *Trends Ecol. Evol.* 20, 387–394.  
<https://doi.org/10.1016/j.tree.2005.04.025>
- Buisson, E., Alvarado, S.T., Le Stradic, S., Morellato, L.P.C., 2017. Plant phenological research enhances ecological restoration. *Restor. Ecol.* 25, 164–171.  
<https://doi.org/10.1111/rec.12471>
- Buisson, E., Le Stradic, S., Silveira, F.A.O., Durigan, G., Overbeck, G.E., Fidelis, A., Fernandes, G.W., Bond, W.J., Hermann, J.-M., Mahy, G., Alvarado, S.T., Zaloumis, N.P., Veldman, J.W., 2019. Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. *Biol. Rev.* 94, 590–609.  
<https://doi.org/10.1111/brv.12470>
- Bullock, J.M., Moy, I.L., 2004. Plants as seed traps: Inter-specific interference with dispersal. *Acta Oecologica*. <https://doi.org/10.1016/j.actao.2003.10.005>
- Burkart, A.E., 1969. *Flora ilustrada de Entre Rios (Argentina)*, Instituto Nacional de Tecnología Agropecuaria. Buenos Aires.
- Campbell, C.S., 1985. The subfamilies and tribes of Gramineae (Poaceae) in the southeastern United States. *J. Arnold Arboretum*. 66, 123–199. <https://doi.org/10.5962/bhl.part.13181>
- Cantero, J.J., Partel, M., Zobel, M., 1999. Is Species Richness Dependent on the Neighbouring Stands? An Analysis of the Community Patterns in Mountain Grasslands of Central Argentina. *Oikos* 87, 346–354. <https://doi.org/10.2307/3546750>
- Chabrierie, O., Alard, D., 2005. Comparison of three seed trap types in a chalk grassland: Toward a standardised protocol. *Plant Ecol.* <https://doi.org/10.1007/s11258-004-0024-2>
- Clinebell, R.R., 2005. Tips for gathering individual species, in: Stephen Packard, C.F.M. (Ed.), *The Tallgrass Restoration Handbook*. Island press, pp. 127–134.
- Coiffait-Gombault, C., Buisson, E., Dutoit, T., 2011. Hay Transfer Promotes Establishment of Mediterranean Steppe Vegetation on Soil Disturbed by Pipeline Construction. *Restor. Ecol.* 19, 214–222. <https://doi.org/10.1111/j.1526-100X.2010.00706.x>
- Cosyns, E., Claerbout, S., Lamoot, I., Hoffmann, M., 2005. Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecol.* 178, 149–162.  
<https://doi.org/10.1007/s11258-004-2846-3>
- Cruz, P., De Quadros, F.L.F., Theau, J.P., Frizzo, A., Jouany, C., Duru, M., Carvalho, P.C.F., 2010. Leaf traits as functional descriptors of the intensity of continuous grazing in native grasslands in the South of Brazil. *Rangel. Ecol. Manag.* 63, 350–358.  
<https://doi.org/10.2111/08-016.1>
- da Silva Menezes, L., Vogel Ely, C., Lucas, D.B., Minervini Silva, G.H., Boldrini, I.I., Overbeck, G.E., 2018. Plant species richness record in Brazilian Pampa grasslands and implications. *Rev. Bras. Bot.* 41, 817–823. <https://doi.org/10.1007/s40415-018-0492-6>
- de Souza Vieira, M., Overbeck, G.E., 2020. Small seed bank in grasslands and tree plantations in former grassland sites in the South Brazilian highlands. *Biotropica*. <https://doi.org/10.1111/btp.12785>
- Deble, L.P., Marchiori, J.N.C., 2014. Sinopse do gênero *Gamochaeta* Weddel (Asteraceae-Gnaphalieae) no Brasil. *Balduinia* 0, 21–31. <https://doi.org/10.5902/2358198014040>
- Díaz, S., Acosta, A., Cabido, M., Diaz, S., 1994. Grazing and the Phenology of Flowering and Fruiting in a Montane Grassland in Argentina: A Niche Approach. *Oikos* 70, 287.  
<https://doi.org/10.2307/3545640>

- Duarte, E., Aona-Pinheiro, Lidyanne Costa, G., Bezerra, C., Soares de Oliveira Neto, J., 2019. Glossário eletrônico de morfologia vegetal: sementes e propágulos [WWW Document]. Univ. Fed. Goiás/ Univ. Fed. do Recôncavo da Bahia. URL [www.projetosbotanica.icb.ufg.br/guia\\_sd/glossary](http://www.projetosbotanica.icb.ufg.br/guia_sd/glossary) (accessed 11.18.20).
- Fedrigo, J.K., Ataíde, P.F., Filho, J.A., Oliveira, L. V., Jaurena, M., Laca, E.A., Overbeck, G.E., Nabinger, C., 2018. Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. *Restor. Ecol.* 26, 677–685. <https://doi.org/10.1111/rec.12635>
- Feldman, S.R., Alzugaray, C., Lewis, J.P., 2007. Relación entre la vegetación y el banco de semillas de un espartillar de *Spartina argentinensis*. *Cienc. e Investig. Agrar.* 34, 41–48. <https://doi.org/10.4067/s0718-16202007000100005>
- Ferreira, P.M.A., Andrade, B.O., Podgaiski, L.R., Dias, A.C., Pillar, V.D., Overbeck, G.E., Milton de, S.M., Boldrini, I.I., 2020. Long-term ecological research in southern Brazil grasslands: Effects of grazing exclusion and deferred grazing on plant and arthropod communities. *PLoS One* 15, 1–23. <https://doi.org/10.1371/journal.pone.0227706>
- Fetzel, T., Havlik, P., Herrero, M., Erb, K.H., 2017. Seasonality constraints to livestock grazing intensity. *Glob. Chang. Biol.* 23, 1636–1647. <https://doi.org/10.1111/gcb.13591>
- Fidelis, A., Blanco, C., 2014. Does fire induce flowering in Brazilian subtropical grasslands? *Appl. Veg. Sci.* 17, 690–699. <https://doi.org/10.1111/avsc.12098>
- Fischer, F.M., Bonnet, O.J.F., Cezimbra, I.M., Pillar, V.D., 2019. Long-term effects of grazing intensity on strategies and spatial components of functional diversity in subtropical grassland. *Appl. Veg. Sci.* 22, 39–47. <https://doi.org/10.1111/avsc.12402>
- Flora do Brasil 2020, 2020. Flora do Brasil 2020 [WWW Document]. Jard. Botânico do Rio Janeiro. URL [www.floradobrasil.jbrj.gov.br](http://www.floradobrasil.jbrj.gov.br) (accessed 11.12.20).
- Funes, G., Basconcelo, S., Díaz, S., Cabido, M., 2001. Edaphic patchiness influences grassland regeneration from the soil seed-bank in mountain grasslands of central Argentina. *Austral Ecol.* 26, 205–212. <https://doi.org/10.1046/j.1442-9993.2001.01102.x>
- Galíndez, G., Ortega-Baes, P., Scopel, A.L., Hutchings, M.J., 2013. The dynamics of three shrub species in a fire-prone temperate savanna: The interplay between the seed bank, seed rain and fire regime. *Plant Ecol.* 214, 75–86. <https://doi.org/10.1007/s11258-012-0147-9>
- Gasparino, D., Malavasi, U.C., Malavasi, M. de M., Souza, I. de, 2006. Quantificação do banco de sementes sob diferentes usos do solo em área de domínio ciliar. *Rev. Árvore* 30, 1–9. <https://doi.org/10.1590/s0100-67622006000100001>
- Gonnet, J.M., 2001. Influence of cattle grazing on population density and species richness of granivorous birds (Emberizidae) in the arid plain of the Monte, Argentina. *J. Arid Environ.* 48, 569–579. <https://doi.org/10.1006/jare.2000.0777>
- Grime, J.P., 1979. *Plant strategies and vegetation process*, 1 st. ed. John Wiley and Sons, Chichester.
- Han, J., Chen, J., Xia, J., Li, L., 2015. Grazing and watering alter plant phenological processes in a desert steppe community. *Plant Ecol.* 216, 599–613. <https://doi.org/10.1007/s11258-015-0462-z>
- Haretche, F., Rodríguez, C., 2006. Banco de semillas de un pastizal uruguayo bajo diferentes condiciones de pastoreo. *Ecol. Austral* 16, 105–113.
- Janzen, D.H., 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. *Am. Nat.*

<https://doi.org/10.1086/284208>

- Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L., Hölzel, N., 2010. Species introduction in restoration projects - Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic Appl. Ecol.* 11, 285–299. <https://doi.org/10.1016/j.baae.2009.12.004>
- Kiehl, K., Wagner, C., 2006. Effect of Hay Transfer on Long-Term Establishment of Vegetation and Grasshoppers on Former Arable Fields. *Restor. Ecol.* 14, 157–166. <https://doi.org/10.1111/j.1526-100X.2006.00116.x>
- Kinucan, R.J., Smeins, F.E., 1992. Soil Seed Bank of a Semiarid Texas Grassland Under Three Long-Term (36- Years) Grazing Regimes. *Am. Midl. Nat.* 128, 11–21. <https://doi.org/10.2307/2426408>
- Kolb, R.M., Pilon, N.A.L., Durigan, G., 2016. Factors influencing seed germination in Cerrado grasses . *Acta Bot. Bras.* .
- Kollmann, J., Goetze, D., 1998. Notes on seed traps in terrestrial plant communities. *Flora* 193, 31–40. [https://doi.org/10.1016/S0367-2530\(17\)30813-7](https://doi.org/10.1016/S0367-2530(17)30813-7)
- Le Stradic, S., Buisson, E., Fernandes, G.W., 2014. Restoration of Neotropical grasslands degraded by quarrying using hay transfer. *Appl. Veg. Sci.* 17, 482–492. <https://doi.org/10.1111/avsc.12074>
- Le Stradic, S., Buisson, E., Fernandes, G.W., Morellato, L.P.C., 2018. Reproductive phenology of two co-occurring Neotropical mountain grasslands. *J. Veg. Sci.* 29, 15–24. <https://doi.org/10.1111/jvs.12596>
- Lemon, A.J., Bolker, B., Oom, S., Klein, E., Rowlingson, B., Wickham, H., Tyagi, A., Eterradosi, O., Grothendieck, G., Toews, M., Kane, J., Turner, R., Witthoft, C., Stander, J., Petzoldt, T., Duursma, R., Biancotto, E., Levy, O., Dutang, C., Solymos, P., Engelmann, R., Hecker, M., Steinbeck, F., Borchers, H., Singmann, H., Toal, T., Ogle, D., Lemon, M.J., 2016. Package ‘plotrix.’
- Lennartsson, T., Wissman, J., Bergström, H.M., 2012. The effect of timing of grassland management on plant reproduction. *Int. J. Ecol.* 2012. <https://doi.org/10.1155/2012/156274>
- Leon, R.J.C., Bertiller, M., 1982. Aspectos fenológicos de dos comunidades del pastizal de la Depresion del Salado. *Bol. la Soc. Argentina Bot.* 20, 329–348.
- Lezama, F., Baeza, S., Altesor, A., Cesa, A., Chaneton, E.J., Paruelo, J.M., 2014. Variation of grazing-induced vegetation changes across a large-scale productivity gradient. *J. Veg. Sci.* <https://doi.org/10.1111/jvs.12053>
- Li, Yu, Dong, S., Gao, Q., Zhang, Y., Liu, S., Swift, D., Ganjurjav, H., Hu, G., Wang, X., Yan, Y., Wu, H., Luo, W., Ge, Y., Li, Yan, Zhao, Z., Gao, X., Li, S., Song, J., 2019. The effects of grazing regimes on phenological stages, intervals and divergences of alpine plants on the Qinghai-Tibetan Plateau. *J. Veg. Sci.* 30, 134–145. <https://doi.org/10.1111/jvs.12703>
- Linabury, M.C., Turley, N.E., Brudvig, L.A., 2019. Insects remove more seeds than mammals in first-year prairie restorations. *Restor. Ecol.* <https://doi.org/10.1111/rec.13004>
- Londo, G., 1976. The decimal scale for relevés of permanent quadrats. *Vegetatio.* <https://doi.org/10.1007/BF00055300>
- Loydi, A., Zalba, S.M., Distel, R.A., 2012. Viable seed banks under grazing and enclosure conditions in montane mesic grasslands of Argentina. *Acta Oecologica* 43, 8–15.

<https://doi.org/10.1016/j.actao.2012.05.002>

- Lucero, J.E., Callaway, R.M., 2018. Granivory from native rodents and competition from an exotic invader strongly and equally limit the establishment of native grasses. *Oecologia*. <https://doi.org/10.1007/s00442-018-4085-7>
- Maarel, E., Sykes, M.T., 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *J. Veg. Sci.* 4, 179–188. <https://doi.org/10.2307/3236103>
- Marone, L., Rossi, B.E., De Casenave, J.L., 1998. Granivore impact on soil-seed reserves in the central Monte desert, Argentina. *Funct. Ecol.* 12, 640–645. <https://doi.org/10.1046/j.1365-2435.1998.00226.x>
- Márquez, S., Funes, G., Cabido, M., Pucheta, E., Multidisciplinario, I., Vegetal, D.B., Cátedra, I., Geofísica, D. De, Biología, A., Exactas, F.D.C., Naturales, F., Nacional, U., Juan, D.S., Juan-argentina, S., 2002. Efectos del pastoreo sobre el banco de semillas germinable y la vegetación establecida en pastizales de montaña del centro de Argentina. *Grazing effects on the germinable seed bank and standing vegetation in mountain grasslands from central Argentina* El pa 327–337.
- Marteinsdóttir, B., 2014. Seed rain and seed bank reveal that seed limitation strongly influences plant community assembly in grasslands. *PLoS One* 9, e103352. <https://doi.org/10.1371/journal.pone.0103352>
- Martínková, J., Smilauer, P., Mihulka, S., 2002. Phenological pattern of grassland species: relation to the ecological and morphological traits. *Flora - Morphol. Distrib. Funct. Ecol. Plants* 197, 290–302. <https://doi.org/https://doi.org/10.1078/0367-2530-00043>
- Milchunas, D.G., Sala, O.E., Lauenroth, W.K., 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* 132, 87–106. <https://doi.org/10.1086/284839>
- Minervini-Silva, G.H., Overbeck, G.E., 2020. Soil seed bank in a subtropical grassland under different grazing intensities. *Acta Bot. Brasilica* 34, 360–370. <https://doi.org/10.1590/0102-33062019abb0297>
- Minervini Silva, G.H., Overbeck, G.E., 2021. Seasonal patterns of endozoochory by cattle in subtropical grassland in southern Brazil. *Austral Ecol. aec.13054*. <https://doi.org/10.1111/aec.13054>
- Morellato, L.P.C., Alberti, L.F., Hudson, I.L., 2010. Applications of circular statistics in plant phenology: A case studies approach, in: *Phenological Research: Methods for Environmental and Climate Change Analysis*. Springer Netherlands, pp. 339–359. [https://doi.org/10.1007/978-90-481-3335-2\\_16](https://doi.org/10.1007/978-90-481-3335-2_16)
- Müller, J., Heinze, J., Joshi, J., Boch, S., Klaus, V.H., Fischer, M., Prati, D., 2014. Influence of experimental soil disturbances on the diversity of plants in agricultural grasslands. *J. Plant Ecol.* 7, 509–517. <https://doi.org/10.1093/jpe/rtt062>
- Nabinger, C., Ferreira, E.T., Freitas, A.K., Carvalho, P.C.F., Sant’Anna, D.M., 2009. Produção animal com base no campo nativo: aplicações de resultados de pesquisa., in: *Campos Sulinos: Conservação e Uso Sustentável Da Biodiversidade*.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan: Community Ecology Package*. R package version 2.4-2. *Community Ecol. Packag.*

- Oleques, S.S., Overbeck, G.E., de Avia, R.S., 2017. Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. *Flora Morphol. Distrib. Funct. Ecol. Plants* 229, 141–146. <https://doi.org/10.1016/j.flora.2017.02.024>
- Oleques, S.S., Vizontin-Bugoni, J., Overbeck, G.E., 2019. Influence of grazing intensity on patterns and structuring processes in plant–pollinator networks in a subtropical grassland. *Arthropod. Plant. Interact.* 13, 757–770. <https://doi.org/10.1007/s11829-019-09699-8>
- Overbeck, G.E., Hermann, J.M., Andrade, B.O., Boldrini, I.I., Kiehl, K., Kirmer, A., Koch, C., Kollmann, J., Meyer, S.T., Müller, S.C., Nabinger, C., Pilger, G.E., Trindade, J.P.P., Vélez-Martin, E., Walker, E.A., Zimmermann, D.G., Pillar, V.D., 2013. Restoration ecology in Brazil-time to step out of the forest. *Nat. a Conserv.* 11, 92–95. <https://doi.org/10.4322/natcon.2013.015>
- Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C., Boldrini, I.I., Both, R., Forneck, E., 2007. Brazil’s neglected biome: The South Brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* 9, 101–116. <https://doi.org/10.1016/j.ppees.2007.07.005>
- Overbeck, G.E., Müller, S.C., Pillar, V.D., Pfadenhauer, J., 2005. Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *J. Veg. Sci.* 16, 655–664. <https://doi.org/10.1111/j.1654-1103.2005.tb02408.x>
- Palma, A.C., Laurance, S.G.W., 2015. A review of the use of direct seeding and seedling plantings in restoration: What do we know and where should we go? *Appl. Veg. Sci.* 18, 561–568. <https://doi.org/10.1111/avsc.12173>
- Pasini, E., Katinas, L., Ritter, M.R., 2014. O gênero *Chaptalia* ( Asteraceae , Mutisieae ) no Rio Grande do Sul , Brasil. *Rodriguésia* 65, 141–158.
- Peters, D.P.C., 2000. Climatic variation and simulated patterns in seedling establishment of two dominant grasses at a semi-arid-arid grassland ecotone. *J. Veg. Sci.* <https://doi.org/10.2307/3246579>
- Pillar, V.D.P., 2006. User s Guide v. 2.4. Memory.
- Pinheiro, M., Abrão, B.E. de, Harter-Marques, B., Miotto, S.T.S., 2008. Floral resources used by insects in a grassland community in Southern Brazil. *Rev. Bras. Botânica* 31. <https://doi.org/10.1590/s0100-84042008000300011>
- R Core Team (2020), 2020. R: A language and environment for statistical computing. R A Lang. Environ. Stat. Comput. R Found. Stat. Comput. Vienna, Austria.
- R Core Team 2019, 2019. : A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL <http://www.R-project.org/>.
- Roberts, H.A., 1981. Seed banks in soils. *Adv. Appl. Biol.*
- Sale, P.F., 1977. Maintenance of High Diversity in Coral Reef Fish Communities. *Am. Nat.* 111, 337–359. <https://doi.org/10.1086/283164>
- Shmida, A., Ellner, S., 1984. Coexistence of plant species with similar niches. *Vegetatio* 58, 29–55. <https://doi.org/10.1007/BF00044894>
- Silva, I.A., Guido, A., Müller, S.C., 2020. Predicting plant performance for the ecological restoration of grasslands: the role of regenerative traits. *Restor. Ecol.* <https://doi.org/10.1111/rec.13182>
- Stucky, B.J., Guralnick, R., Deck, J., Denny, E.G., Bolmgren, K., Walls, R., 2018. The plant phenology ontology: A new informatics resource for large-scale integration of plant

- phenology data. *Front. Plant Sci.* 9, 1–12. <https://doi.org/10.3389/fpls.2018.00517>
- Thomas, P., Overbeck, G., Müller, S., 2019. Restoration of abandoned subtropical highland grasslands in Brazil: mowing produces fast effects, but hay transfer does not, *Acta Botanica Brasilica*. <https://doi.org/10.1590/0102-33062018abb0377>
- Thompson, K., Bakker, J.P., Bekker, R.M., 1997. Seed bank classification, in: *The Soil Seed Banks of North West Europe: Methodology, Density and Longevity*. Cambridge University Press, Melbourne, p. 276.
- Török, P., Migléc, T., Valkó, O., Kelemen, A., Tóth, K., Lengyel, S., Tóthmérész, B., 2012. Fast restoration of grassland vegetation by a combination of seed mixture sowing and low-diversity hay transfer. *Ecol. Eng.* 44, 133–138. <https://doi.org/10.1016/j.ecoleng.2012.03.010>
- Veldman, J.W., Buisson, E., Durigan, G., Fernandes, G.W., Le Stradic, S., Mahy, G., Negreiros, D., Overbeck, G.E., Veldman, R.G., Zaloumis, N.P., Putz, F.E., Bond, W.J., 2015. Toward an old-growth concept for grasslands, savannas, and woodlands. *Front. Ecol. Environ.* 13, 154–162. <https://doi.org/10.1890/140270>
- Vieira, M. de S., Bonilha, C.L., Boldrini, I.I., Overbeck, G.E., 2015. The seed bank of subtropical grasslands with contrasting land-use history in southern Brazil. *Acta Bot. Brasilica* 29, 543–552. <https://doi.org/10.1590/0102-33062015abb0026>
- Vogel Ely, C., 2019. Sistemática e conservação das espécies sul-americanas não andinas de *Hypericum L.* (HYPERICACEAE). Fed. Univ. Rio Gd. do Sul. Federal University of Rio Grande do Sul.
- von Blanckenhagen, B., Poschod, P., 2005. Restoration of calcareous grasslands: The role of the soil seed bank and seed dispersal for recolonisation processes. *Biotechnol. Agron. Soc. Environ.* BASE 9, 143–149.
- Wagner, M., Hulmes, S., Hulmes, L., Redhead, J.W., Nowakowski, M., Pywell, R.F., 2020. Green hay transfer for grassland restoration: species capture and establishment. *Restor. Ecol.* 1–11. <https://doi.org/10.1111/rec.13259>
- Wilson, J.B., Peet, R.K., Dengler, J., Pärtel, M., 2012. Plant species richness: The world records. *J. Veg. Sci.* 23, 796–802. <https://doi.org/10.1111/j.1654-1103.2012.01400.x>
- Zar, J., 2010. *Biostatistical analysis*. 5th, Pearson Prentice-Hall, Upper Saddle River.
- Zarco, A., Cueto, V.R., Sagario, M.C., Marone, L., 2019. Effects of livestock grazing on flocks of seed-eating birds in the central monte desert, Argentina. *Can. J. Zool.* <https://doi.org/10.1139/cjz-2018-0223>
- Zhu, J., Zhang, Y., Liu, Y., 2016. Effects of short-term grazing exclusion on plant phenology and reproductive succession in a Tibetan alpine meadow. *Sci. Rep.* 6, 2–10. <https://doi.org/10.1038/srep27781>
- Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. *Analyzing Ecological Data, Methods*. <https://doi.org/10.1016/B978-0-12-387667-6.00013-0>



## Supporting Information

**Table S1.** Species density (seedlings/m<sup>2</sup>) found in seed rain for each grazing intensity (% of forage allowance) and ungrazed (UG) treatments and seasons. Values: mean of two replicas for each intensity grazing and ungrazed treatments.

Family/ Species	Summer					Autumn					Winter					Spring					
	4%	8%	12%	16%	UG	4%	8%	12%	16%	UG	4%	8%	12%	16%	UG	4%	8%	12%	16%	UG	
<b>Acanthaceae</b>																					
<i>Ruellia morongii</i> Britton		38.52	57.77	38.52				38.52										38.52		19.26	
<b>Apiaceae</b>																					
<i>Centella asiatica</i> (L.) Urb.	115.55		38.52							38.52	19.26	134.80		19.26	38.52						
<i>Cyclospermum</i> <i>leptophyllum</i> (Pers.) Sprague	57.77		19.26		19.26																
<i>Eryngium</i> <i>horridum</i> Malme			38.52	38.52														192.58	442.92	96.29	327.38
<b>Araliaceae</b>																					
<i>Hydrocotyle</i> <i>exigua</i> Malme	519.95			19.26	19.26					19.26						19.26	19.26	38.52			
<b>Asteraceae</b>																					
<i>Baccharis</i> <i>coridifolia</i> DC.					57.77																
<i>Baccharis</i> <i>crispa</i> Spreng.		192.58	38.52	192.58	134.80		38.52	57.77	19.26	327.38	19.26	38.52								57.77	
<i>Baccharis</i> <i>dracunculifolia</i> DC.	19.26	19.26	19.26	19.26	6,258.70	500.70			19.26	2,349.42				19.26	96.29		19.26			231.09	



<i>Chaptalia piloselloides</i> (Vahl) Baker	19.26			19.26															
<i>Chaptalia runcinata</i> Kunth	19.26			38.52					19.26										
<i>Chevreulia acuminata</i> Less.	19.26										38.52		19.26	19.26					
<i>Chevreulia sarmentosa</i> (Pers.) S.F.Blake	19.26								173.32	154.06		38.52	57.77	19.26		19.26			
<i>Chromolaena congesta</i> (Hook. & Arn.) R.M.King & H.Rob.	19.26		115.55	423.67					616.24								19.26		
<i>Conyza bonariensis</i> (L.) Cronquist		38.52	96.29		19.26					19.26		19.26							19.26
<i>Gamochaeta americana</i> (Willd.) Wedd.				134.80	19.26				57.77	19.26	19.26	19.26	19.26	19.26	38.52				19.26
<i>Gamochaeta calviceps</i> (Fernald) Cabrera				57.77					77.03		38.52		19.26						
<i>Gamochaeta coarctata</i> (Willd.) Kerguelen	96.29	38.52	57.77		57.77	115.55	134.80	134.80	134.80	423.67	770.30	539.21	539.21	539.21	154.06	308.12	19.26	38.52	231.09
<i>Gamochaeta pensylvanica</i> (Willd.) Cabrera							19.26		77.03	96.29	96.29	38.52	19.26	19.26	134.80	19.26	38.52	77.03	
<i>Micropsis spathulata</i> (Pers.) Cabrera	19.26								38.52		19.26			38.52			38.52		

<i>Pterocaulon angustifolium</i> DC.	96.29	96.29		134.80	38.52	57.77	19.26	115.55		38.52										19.26
<i>Senecio brasiliensis</i> (Spreng.) Less.																				19.26
<i>Senecio madagascariensis</i> Poir.										19.26	154.06									
<i>Solidago chilensis</i> Meyen				19.26																
<i>Soliva sessilis</i> Ruiz & Pav.	38.52													19.26	57.77					19.26
<i>Stenachaenium campestre</i> Baker					19.26															
<i>Stevia sp.</i> Cav.					231.09	19.26		19.26		57.77										
<i>Vernonanthura nudiflora</i> (Less.) H.Rob.	38.52						38.52	19.26			38.52	19.26								
<b>Brassicaceae</b>																				
<i>Cardamine chenopodiifolia</i> Pers.	19.26	192.58	250.35	77.03	192.58	1,116.94	1,348.03	982.13	885.85	770.30	231.09	211.83	404.41	173.32	308.12	115.55	96.29	77.03	115.55	211.83
<b>Campanulaceae</b>																				
<i>Lobelia hederacea</i> Cham.	19.26																			
<b>Convolvulaceae</b>																				
<i>Dichondra sericea</i> Sw.										19.26				38.52	19.26					
<b>Cyperaceae</b>																				

<i>Bulbostylis subtilis</i> M.G. López	19.26	19.26	19.26		19.26		19.26	19.26		38.52
<i>Carex sororia</i> Kunth								38.52		173.32 19.26
<i>Cyperus aggregatus</i> (Willd.) Endl.		19.26	19.26		19.26			19.26		
<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.			19.26		38.52 38.52			19.26 57.77		96.29 115.55
<i>Eleocharis viridans</i> Kük. ex Osten	19.26	57.77	19.26		38.52		19.26	38.52		
<i>Fimbristylis autumnalis</i> (L.) Roem. & Schult.	19.26				19.26					38.52
<i>Fimbristylis dichotoma</i> (L.) Vahl	96.29		77.03				19.26		19.26	
<i>Rhynchospora Barrosiana</i> Guagl.		38.52								
<i>Rhynchospora tenuis</i> Link	19.26		19.26					38.52	19.26	77.03
<b>Fabaceae</b>										
<i>Desmanthus virgatus</i> (L.) Willd.	19.26									

<i>Desmodium adscendens</i> (Sw.) DC.																		19.26	
<i>Stylosanthes leiocarpa</i> Vogel																		19.26	
<b>Hypericaceae</b>																			
<i>Hypericum caprifoliatum</i> Cham. & Schltdl.		19.26		1,271.00						654.76								481.44	
<b>Hypoxidaceae</b>																			
<i>Hypoxis decumbens</i> L.	38.52	19.26	38.52	57.77	134.80	38.52				19.26	19.26	19.26	19.26	96.29	115.55			38.52	
<b>Iridaceae</b>	57.77		38.52							38.52	38.52	19.26						38.52	19.26
<i>Sisyrinchium micranthum</i> Cav.				19.26															
<i>Sisyrinchium ostenianum</i> Beauverd						19.26				115.55								57.77	
<i>Sisyrinchium vaginatum</i> Spreng.						19.26				115.55								57.77	
<b>Juncaceae</b>																			
<i>Juncus capillaceus</i> Lam.																		19.26	
<i>Juncus microcephalus</i> Kunth										19.26									
<b>Malvaceae</b>																			

<i>Krapovickasia flavescens</i> (Cav.) Fryxell																					19.26
<b>Oxalidaceae</b>																					
<i>Oxalis brasiliensis</i> G. Lodd.				19.26						96.29	38.52	19.26	96.29								
<i>Oxalis conorrhiza</i> Jacq.	57.77	38.52	19.26	57.77	19.26	77.03	173.32	96.29	96.29	134.80	250.35	308.12	154.06	134.80	173.32	38.52	115.55	173.32	19.26	96.29	
<i>Oxalis eriocarpa</i> DC.				19.26					19.26			19.26									
<b>Plantaginaceae</b>																					
<i>Mecardonia procumbens</i> var. <i>tenella</i> (Cham. & Schltdl.) V.C.Souza	19.26				77.03					38.52						19.26	19.26				173.32
<i>Plantago myosurus</i> Lam.	19.26							19.26		38.52	19.26					173.32	19.26				19.26
<i>Plantago tomentosa</i> Lam.					38.52												38.52	19.26	19.26		38.52
<b>Poaceae</b>																					
<i>Andropogon lateralis</i> Nees				19.26																	19.26
<i>Andropogon selloanus</i> (Hack.) Hack.				19.26																	
<i>Axonopus affinis</i> Chase	654.76	38.52	154.06	115.55		57.77	77.03	173.32	96.29		19.26					211.83	19.26	38.52			38.52
<i>Dichantherium sabulorum</i> (Lam.)		19.26	19.26	96.29	19.26				96.29	19.26	19.26							38.52	38.52		57.77

Gould & C.A.Clark															
<i>Digitaria ciliaris</i> (Retz.) Koeler			57.77												
<i>Digitaria violascens</i> Link	19.26	19.26	38.52	96.29		19.26	19.26		19.26		77.03	57.77	308.12	38.52	115.55
<i>Eleusine tristachya</i> (Lam.) Lam.	57.77		19.26		38.52			38.52	19.26		192.58				
<i>Eragrostis lugens</i> Nees	38.52	57.77	77.03	19.26	115.55	96.29	134.80	346.64	19.26		19.26	38.52			19.26
<i>Eragrostis neesii</i> Trin.		19.26				19.26			77.03	19.26	173.32				57.77
<i>Lolium multiflorum</i> Lam.															19.26
<i>Mnesithea selloana</i> (Hack.) de Koning & Sosef	77.03	19.26	77.03	19.26											
<i>Panicum beergi</i> Arechav.												19.26			
<i>Paspalum notatum</i> Flügge															19.26
<i>Paspalum pumilum</i> Nees		77.03		134.80	19.26				57.77						
<i>Piptochaetium montevidense</i> (Spreng.) Parodi	19.26	19.26			19.26	19.26	38.52		77.03	38.52	19.26		19.26	19.26	
Poaceae sp. 1	19.26														
Poaceae sp. 2	38.52														

<i>Sacciolepis vilvoidea</i> Chase																			19.26		19.26		
<i>Schizachyrium microstachyum</i> (Desv. ex Ham.) Roseng., B.R. Arrill. & Izag.			57.77		19.26					19.26													
<i>Setaria parviflora</i> (Poir.) M. Kerguelen	77.03	134.80	96.29		19.26					19.26		19.26		38.52	19.26						38.52	19.26	
<i>Sporobolus indicus</i> (L.) R.Br.																					38.52	19.26	
<i>Steinchisma hians</i> (Elliott) Nash	57.77	38.52	346.64	38.52	134.80	231.09	115.55	57.77		38.52	38.52	19.26	19.26	19.26	346.64	38.52	134.80	134.80					
<b>Polygalaceae</b>																							
<i>Polygala australis</i> A.W. Benn.																					19.26	19.26	
<b>Primulaceae</b>																							
<i>Lysimachia minima</i> (L.) U. Manns & Anderb.												19.26	134.80								19.26	57.77	19.26
<b>Rubiaceae</b>																							
<i>Borreria dasycephala</i> (Cham. & Schlttdl.) Bacigalupo & E.L.Cabral					19.26																		
<i>Galianthe fastigiata</i> Griseb.																						96.29	

<i>Galium hirtum</i> Lam.																			19.26
<i>Galium humile</i> Cham. & Schltdl.				38.52	38.52	192.58	19.26	38.52		19.26									19.26
<i>Galium richardianum</i> (Gillies ex Hook. & Arn.) Endl. ex Walp.	38.52	19.26	57.77			115.55		19.26	19.26		38.52	57.77							19.26
<i>Richardia humistrata</i> (Cham. & Schltdl.) Steud.			173.32	77.03			19.26		19.26									96.29	250.35
<i>Richardia stellaris</i> (Cham. & Schltdl.) Steud.	19.26			19.26				96.29			19.26								57.77
<i>Spermacoce verticillata</i> L.	19.26							38.52											38.52
<b>Solanaceae</b>																			
<i>Solanum viarum</i> Dunal								19.26		77.03	57.77			19.26	19.26				
<b>Verbenaceae</b>																			
<i>Glandularia marrubioides</i> (Cham.) Tronc.	19.26							19.26											



**Table S2.** Results of randomization tests (MULTIV, Pillar 1999) comparing density (seedlings/m<sup>2</sup>), functional groups (C3 grasses, C4 grasses, monocots, dicots, subshrubs), life cycle (non-perennials, perennials), structures adapted to dispersal (awns, wings, trichomes, pappus) and Sørensen index (similarity between seed rain and established vegetation) among grazing intensity/ungrazed treatments and seasons. The symbol \* indicates significant differences (P≤0.05). Qb: sums of squares; FA: forage allowance.

Source of variation	Density (seedlings/m <sup>2</sup> )		C3 grasses		C4 grasses		Monocots		Dicots		Subshrubs			
	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value		
Summer - Autumn	5.74E+06	0.665	1.56E+04	0.78	1.38E+06	0.213	39,869	0.677	2.42E+07	0.026*	3.38E+07	0.178		
Summer - Winter	1.16E+08	0.158	4.21E+05	0.020*	9.27E+06	0.001*	4.88E+05	0.059*	1.72E+07	0.029*	1.09E+08	0.091		
Summer - Spring	8.48E+07	0.113	1.56E+04	0.746	1.05E+06	0.201	3.59E+05	0.204	1.21E+06	0.512	9.97E+07	0.0009*		
Autumn - Winter	6.99E+07	0.047*	2.75E+05	0.004*	3.50E+06	0.0009*	2.49E+05	0.118	5.99E+05	0.771	2.16E+07	0.144		
Autumn - Spring	4.64E+07	0.065	6.23E+04	0.564	22,426	0.871	6.38E+05	0.055*	1.46E+07	0.045	1.74E+07	0.018*		
Winter - Spring	2.39E+06	0.681	5.99E+05	0.007*	4.09E+06	0.0006*	1.68E+06	0.0004*	9.27E+06	0.054*	2.25E+05	0.895		
4% FA - 8% FA	5.11E+06	0.459	38,155	0.843	2.44E+06	0.0486*	49,836	0.648	7,008.20	0.988	5.68E+05	0.511		
4% FA - 12% FA	8.42E+06	0.311	7,008	0.856	5.68E+05	0.389	1.12E+05	0.328	1.07E+06	0.569	7.48E+05	0.352		
4% FA - 16% FA	1.61E+07	0.159	94,221	0.334	7.97E+05	0.324	12,459	0.847	5.36E+06	0.154	4.12E+05	0.612		
4% FA - Ungrazed	2.83E+08	0.040*	12,459	0.711	3.29E+06	0.0536*	1.99E+05	0.35	2.53E+06	0.541	2.78E+08	0.008*		
8% FA - 12% FA	4.12E+05	0.680*	12,459	0.623	6.55E+05	0.265	12,459	0.723	1.25E+06	0.177	12,459	0.837		
8% FA - 16% FA	3.09E+06	0.243	2.52E+05	0.111	4.49E+05	0.245	1.12E+05	0.346	5.76E+06	0.038*	12,459	0.762		
8% FA - Ungrazed	3.64E+08	0.029*	94,221	0.576	63,073	0.719	4.49E+05	0.06	2.27E+06	0.52	3.04E+08	0.005*		
12% FA - 16% FA	1.25E+06	0.294	1.53E+05	0.402	19,467	0.878	1.99E+05	0.223	1.65E+06	0.091	49,836	0.888		
12% FA - Ungrazed	3.89E+08	0.016*	38,155	0.876	1.12E+06	0.187	6.10E+05	0.062	6.88E+06	0.249	3.08E+08	0.004*		
16% FA - Ungrazed	4.35E+08	0.006*	38,155	0.203	8.48E+05	0.122	1.12E+05	0.483	1.53E+07	0.074	3.00E+08	0.004*		
	Non-perennials		Perennials		Awns		Wings		Trichomes		Pappus		Sørensen index	

Source of variation	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value	Qb	P-value
Summer - Autumn	3.18E+07	0.0003*	6.46E+07	0.051*	622.95	1	22,426	0.009*	2.91E+07	0.262	2.27E+07	0.402	9.77E-03	0.158
Summer - Winter	1.68E+07	0.0005*	2.21E+08	0.006*	22,426	0.298	15,574	0.026*	4.54E+07	0.334	3.59E+07	0.356	1.13E-02	0.2
Summer - Spring	1.44E+06	0.095	1.08E+08	0.051*	5,606.60	0.765	1.75E+06	0.002*	9.09E+07	0.050*	8.44E+07	0.085	1.03E-03	0.656
Autumn - Winter	2.39E+06	0.15	4.64E+07	0.073	30,525	0.121	622.95	1	1.82E+06	0.587	1.50E+06	0.595	5.57E-05	0.93
Autumn - Spring	1.97E+07	0.0009*	5.62E+06	0.544	9,967.20	0.432	2.17E+06	0.001*	1.72E+07	0.14	1.95E+07	0.117	4.46E-03	0.389
Winter - Spring	8.38E+06	0.0036*	1.97E+07	0.013*	5,606.60	0.325	2.10E+06	0.0009*	7.81E+06	0.003*	1.02E+07	0.001*	5.51E-03	0.401
4% FA - 8% FA	1.95E+06	0.184	1.34E+07	0.156	3,114.80	0.635	19,467	1	1.12E+05	0.871	1.12E+05	0.845	3.86E-05	0.944
4% FA - 12% FA	5.26E+05	0.312	1.32E+07	0.139	3,114.80	0.872	3.43E+05	0.333	2.53E+06	0.165	2.62E+06	0.099	4.79E-05	0.954
4% FA - 16% FA	1.32E+05	0.644	1.34E+07	0.122	7,008.20	0.335	7,008.20	0.331	2.36E+06	0.219	1.51E+06	0.339	9.34E-03	0.311
4% FA - Ungrazed	5.68E+05	0.429	2.58E+08	0.028*	3,114.80	0.775	1.12E+05	0.672	2.03E+08	0.032*	2.03E+08	0.029*	1.41E-02	0.202
8% FA - 12% FA	4.49E+05	0.469	778.69	0.994	12,459	0.558	1.99E+05	0.112	1.58E+06	0.083	1.65E+06	0.040*	1.73E-04	0.864
8% FA - 16% FA	3.09E+06	0.078	0	0.998	778.69	1	3,114.80	1	1.44E+06	0.121	7.97E+05	0.23	1.06E-02	0.099
8% FA - Ungrazed	4.12E+05	0.567	3.89E+08	0.020*	12,459	0.328	38155	0.676	2.13E+08	0.046*	2.13E+08	0.043*	1.57E-02	0.041*
12% FA - 16% FA	1.18E+06	0.068	778.69	0.991	19,467	0.502	2.52E+05	0.337	3,114.50	0.963	1.53E+05	0.632	8.05E-03	0.259
12% FA - Ungrazed	778.41	1	3.88E+08	0.005*	3.49E-10	1	63,074	0.443	2.51E+08	0.008*	2.52E+08	0.004*	1.25E-02	0.18
16% FA - Ungrazed	1.25E+06	0.228	3.89E+08	0.008*	19,467	0.251	63,073	0.972	2.49E+08	0.008*	2.40E+08	0.006*	4.96E-04	0.747

**Table S2.** Mean cover value (Londo, 1976), of species recorded in the established vegetation survey for each grazing intensity (% of forage allowance) and ungrazed (UG) treatments and seasons.

Family/ Species	Summer					Autumn					Winter					Spring				
	4%	8%	12%	16%	UG	4%	8%	12%	16%	UG	4%	8%	12%	16%	UG	4%	8%	12%	16%	UG
<b>Acanthaceae</b>																				
<i>Justicia axillaris</i> (Nees) Lindau		0.2	0.1	0.1												0.1	0.1	0.1	0.1	
<i>Ruellia hypericoides</i> (Nees) Lindau	0.2	0.1	0.1	0.2		0.2		0.3									0.1	1.1	0.2	
<i>Ruellia morongii</i> Britton	0.3					0.2					0.1					0.3	0.2			
<b>Amaranthaceae</b>																				
<i>Pfaffia tuberosa</i> (Spreng.) Hicken	0.1	0.1		0.2		0.1	0.1									0.1	0.1			
<b>Amaryllidaceae</b>																				
<i>Nothoscordum bivalve</i> (L.) Britton																		0.1	0.1	0.1
<i>Nothoscordum gracile</i> (Aiton) Stearn											0.1				0.1	0.1	0.1		0.1	0.1
<i>Nothoscordum montevidense</i> Beauverd											0.1				0.1	0.1	0.1			
<i>Zephyranthes americana</i> (Hoffmanns.) Ravenna	0.1																			
<b>Anacardiaceae</b>																				
<i>Schinus terebinthifolius</i> Raddi			0.2																	
<b>Apiaceae</b>																				
<i>Centella asiatica</i> (L.) Urb.	0.5	0.7	0.7	0.1		0.3	1.0	0.2								0.6	1.0		0.2	
<i>Cyclospermum leptophyllum</i> (Pers.) Sprague											0.1					0.1	0.1	0.1		
<i>Eryngium ciliatum</i> Cham. & Schldtl.		0.1	0.1													0.4	0.4	0.4		
<i>Eryngium horridum</i> Malme	4.0	3.3	2.2	1.0		3.3	1.1	2.0	1.0		3.5		3.3		1.0	2.9	1.7	3.6	1.0	
<b>Araliaceae</b>																				

<i>Hydrocotyle exigua</i> Malme										0.1		0.1					0.2		
<b>Aristolochiaceae</b>																			
<i>Aristolochia sessilifolia</i> (Klotzsch) Duch.										0.1								0.2	0.1
<b>Asteraceae</b>																			
<i>Aspilia montevidensis</i> (Spreng.) Kuntze	0.1	0.1	0.2	0.2	0.3	0.1	0.1	0.1		0.3		0.1	3.0	0.4	0.1	0.1		0.1	1.2
<i>Austroeupatorium inulaefolium</i> (Kunth) R.M.King & H.Rob.					0.8					0.9							0.2		0.3
<i>Baccharis anomala</i> DC.					2.0					1.7						2.6			4.0
<i>Baccharis articulata</i> (Lam.) Pers.					2.0					0.8						0.6			
<i>Baccharis coridifolia</i> DC.									0.1									0.1	
<i>Baccharis crispa</i> Spreng.	0.3	1.5	0.7	1.0	0.7	0.2	1.0	0.6	0.9	0.6	0.2	1.8	0.2	1.2	0.3		0.2		
<i>Baccharis dracunculifolia</i> DC.					1.9					1.4					1.2				
<i>Baccharis riograndensis</i> Malag. & J.Vidal		1.0	0.3		0.1					0.2					0.1				0.1
<i>Bidens pilosa</i> Linn										0.1									
<i>Chaptalia exscapa</i> (Pers.) Baker	0.2						0.1	0.1			0.1		0.1	0.1					
<i>Chaptalia integerrima</i> (Vell.) Burkart	0.1																		
<i>Chaptalia piloselloides</i> (Vahl) Baker							0.1		0.1	0.1	0.1		0.1			0.1			
<i>Chaptalia runcinata</i> Kunth	0.1						0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1		0.1	0.1	
<i>Chevreulia acuminata</i> Less.			0.1	0.2						0.1	0.1					0.1	0.1	0.1	0.1
<i>Chevreulia sarmentosa</i> (Pers.) S.F.Blake											0.1	0.2				0.1	0.1	0.4	0.1
<i>Chromolaena congesta</i> (Hook. & Arn.) R.M.King & H.Rob.	0.2	0.1			0.1					0.2							0.1		
<i>Chromolaena laevigata</i> (Lam.) R.M.King & H.Rob.										1.0									
<i>Chrysolaena flexuosa</i> (Sims) H.Rob.	0.1				0.1					0.1					0.1				0.1
<i>Conyza blakei</i> (Cabrera) Cabrera	0.2								0.2										

<i>Conyza bonariensis</i> (L.) Cronquist																	0.6	0.1	0.1
<i>Conyza primulifolia</i> (Lam.) Cuatrec. & Lourteig						0.1				0.1							0.2		
<i>Elephantopus mollis</i> Kunth			0.1	0.1	0.2			0.2	0.1	0.1	0.1		0.2	0.1	0.1				
<i>Facelis retusa</i> (Lam.) Sch. Bip.																		0.1	
<i>Gamochaeta americana</i> (Will.) Wedd.		0.1	0.1	0.2	0.2												0.1	0.1	0.1
<i>Gamochaeta coarctata</i> (Willd.) Kerguelén																	0.1	0.1	0.1
<i>Hypochaeris chillensis</i> L.				0.1													0.1		
<i>Hypochaeris megapotamica</i> Cabrera																	0.1	0.1	
<i>Micropsis spathulata</i> (Pers.) Cabrera												0.1					0.1	0.1	0.1
<i>Noticastrum calvatum</i> (Baker) Cuatrec.						0.2		0.1		0.2									
<i>Pterocaulon angustifolium</i> DC.			0.1		0.2					0.2					0.1	0.1			0.1
<i>Pterocaulon angustifolium</i> DC.		0.2	0.3	0.3	0.1	0.3	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2		0.1	
<i>Senecio brasiliensis</i> (Spreng.) Less.		0.4				0.4				0.2							0.1		
<i>Senecio leptolobus</i> DC.		0.1	0.1		5.0					0.2							0.1	0.2	2.7
<i>Senecio madagascariensis</i> Poir.						0.3				0.1							0.1		
<i>Senecio selloi</i> (Spreng.) DC.												0.1		0.3			0.1		0.3
<i>Soliva sessilis</i> Ruiz & Pav.						0.1				0.1							0.2	0.1	
<i>Stenachaenium campestre</i> Baker		0.1		0.1	0.1												0.3	0.1	0.1
<i>Vernonanthura nudiflora</i> (Less.) H. Rob.		0.2	0.2	0.3	0.4	0.4	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1				
<i>Vernonanthura tweedieana</i> (Baker) H. Rob.						0.4				0.5							0.4		
<b>Campanulaceae</b>																			
<i>Lobelia hederacea</i> Cham.																	0.1		
<i>Wahlenbergia linarioides</i> (Lam.) A. DC.				0.1		0.1						0.1							0.1

<b>Caryophyllaceae</b>														
<i>Cerastium glomeratum</i> Thuill.										0.1			0.1	
<b>Cistaceae</b>														
<i>Helianthemum brasiliense</i> (Lam.) Pers.													0.1	
<b>Convolvulaceae</b>														
<i>Dichondra sericea</i> Sw.										0.1	0.4	0.1		0.1
<i>Evolvulus sericeus</i> Sw.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1			0.1	0.1
<b>Cyperaceae</b>														
<i>Abildgaardia ovata</i> (Burm.f.) Kral	0.1	0.2	0.1			0.1				0.1			0.2	0.1
<i>Bulbostylis sphaerocephala</i> (Boeckeler) Lindm.				0.1										
<i>Bulbostylis subtilis</i> M.G. López	0.1	0.1				0.3	0.1	0.1					0.1	0.1
<i>Carex bonariensis</i> Desf. ex Poir.														0.1
<i>Carex phalaroides</i> Kunth						0.1				0.1	0.1		0.1	0.1
<i>Carex sororia</i> Kunth													0.1	0.1
<i>Cyperus aggregatus</i> (Willd.) Endl.	0.1			0.1										
<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.				0.1			0.1	0.3						0.6
<i>Cyperus lanceolatus</i> Poir.				0.1										
<i>Cyperus obtusatus</i> (J.Presl & C.Presl) Mattf. & Kük.	0.1				0.2		0.1	0.1					0.1	0.1
<i>Cyperus sesquiflorus</i> (Torr.) Mattf. & Kük.	0.1	0.1			0.1		0.1					0.2	0.1	0.1
<i>Eleocharis viridans</i> Kük. ex Osten	0.1	0.1	0.1				0.4						0.2	1.7
<i>Fimbristylis autumnalis</i> (L.) Roem. & Schult.	0.1		0.2					0.1						
<i>Fimbristylis dichotoma</i> (L.) Vahl	0.1	0.2	0.2	0.2		0.1	0.1	0.1	0.1			0.1	0.1	0.1
<i>Rhynchospora Barrosiana</i> Guagl.		0.4	0.2	0.3	0.1		0.1		0.4			0.1		1.2

<i>Rhynchospora setigera</i> (Kunth) Griseb.												0.1					0.1
<i>Rhynchospora tenuis</i> Link	0.7	0.2	0.6	4.5		0.2	0.1	0.3	2.3			0.3	3.0	0.2	0.2	0.2	2.6
<i>Scleria distans</i> Poir.			0.1		0.1			0.1									
<b>Drosereae</b>																	
<i>Drosera communis</i> A.St.-Hil.																	0.1
<b>Euphorbiaceae</b>																	
<i>Euphorbia selloi</i> (Klotzsch & Garcke) Boiss.	0.1	0.1	0.1	0.2			0.1					0.1		0.1	0.1	0.1	0.1
<i>Tragia bahiensis</i> Müll. Arg.	0.1	0.1	0.1	0.1	0.1		0.1		0.1				0.1		0.1	0.1	0.1
<b>Fabaceae</b>																	
<i>Chamaecrista repens</i> (Vogel) H.S. Irwin & Barneby	0.1																0.1
<i>Clitoria nana</i> Benth.				0.4													
<i>Crotalaria hilariana</i> Benth.					0.1												0.1
<i>Desmanthus virgatus</i> (L.) Willd.											0.1						0.1
<i>Desmodium adscendens</i> (Sw.) DC.					0.2					0.2							0.2
<i>Desmodium incanum</i> DC.	0.5	0.3	0.2	0.1	0.1	0.6	0.1	0.2	0.1				0.2	0.2	0.2		
<i>Galactia marginalis</i> Benth.			0.1														0.1 0.1
<i>Macroptilium prostratum</i> (Benth.) Urb.			0.2	0.4													
<i>Pomaria pilosa</i> (Vogel) B.B. Simpson & G.P. Lewis									0.2								
<i>Rhynchosia diversifolia</i> Micheli		0.4															
<i>Stylosanthes leiocarpa</i> Vogel	0.2	0.2	0.1	0.2	0.1	0.2	0.1						0.2	0.1			
<i>Stylosanthes montevidensis</i> Vogel			0.2		0.1			0.1	0.1	0.1							
<i>Trifolium polymorphum</i> Poir.											0.1			0.1		0.1	
<i>Zornia cryptantha</i> Arechav.	0.1				0.1	0.1					0.1			0.1			0.1

<b>Hypoxidaceae</b>																			
<i>Hypoxis decumbens</i> L.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
<b>Iridaceae</b>																			
<i>Herbertia lahue</i> (Molina) Goldblatt																0.1	0.1	0.1	0.1
<i>Herbertia pulchella</i> Sweet																0.1	0.1	0.1	0.1
<i>Sisyrinchium micranthum</i> Cav.	0.1															0.1	0.1	0.1	0.1
<i>Sisyrinchium ostenianum</i> Beauverd																0.1	0.1		0.1
<i>Sisyrinchium palmifolium</i> L.																			0.1
<i>Sisyrinchium vaginatum</i> Spreng.	0.1				0.1					0.1						0.1			0.1
<b>Juncaceae</b>																			
<i>Juncus capillaceus</i> Lam.									0.1										
<b>Lamiaceae</b>																			
<i>Condea fastigiata</i> (Benth.) Harley & J.F.B. Pastore									0.4							0.2			
<i>Ocimum nudicaule</i> Benth.									0.1										
<i>Peltodon longipes</i> Kunth ex Benth.									0.1										
<b>Linaceae</b>																			
<i>Cliococca selaginoides</i> (Lam.) C.M.Rogers & Mildner	0.1								0.1							0.1	0.1	0.1	0.1
<b>Lythraceae</b>																			
<i>Cuphea glutinosa</i> Cham. & Schldtl.									0.1	0.1	0.1	0.1							0.1
<b>Malvaceae</b>																			
<i>Sida rhombifolia</i> L.									0.1										0.1
<b>Melastomataceae</b>																			
<i>Chaetogastra gracilis</i> (Bonpl.) DC.	0.1				0.1	0.1	0.1		0.1							0.1			0.1



<b>Moraceae</b>													
<i>Dorstenia brasiliensis</i> Lam.													0.1
<b>Orchidaceae</b>													
<i>Habenaria parviflora</i> Lindl.													0.1
<i>Prescottia densiflora</i> (Brongn.) Lindl.													0.1
<b>Orobanchaceae</b>													
<i>Agalinis communis</i> (Cham. & Schltdl.) D'Arcy												0.1	
<b>Oxalidaceae</b>													
<i>Oxalis bifrons</i> Progel						0.3		0.1		0.1			
<i>Oxalis bipartita</i> A. St.-Hil.												0.1	
<i>Oxalis brasiliensis</i> G. Lodd.						0.1					0.1	0.1	0.1
<i>Oxalis conorrhiza</i> Jacq.							0.1	0.1	0.1	0.1			0.1
<i>Oxalis eriocarpa</i> DC.						0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Oxalis floribunda</i> Lehm.						0.1	0.1				0.1		
<i>Oxalis lasiopetala</i> Zucc.						0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<i>Oxalis lindneri</i> Knuth										0.1	0.1		0.1
<b>Plantaginaceae</b>													
<i>Cyclosporum leptophyllum</i> (Pers.) Sprague ex Britton & P.Wilson													0.1
<i>Mecardonia procumbens</i> var. <i>tenella</i> (Cham. & Schltdl.) V.C.Souza										0.1	0.1		0.1
<i>Plantago australis</i> Lam.													0.1
<i>Plantago myosurus</i> Lam.						0.1				0.1		0.1	0.1
<i>Plantago penantha</i> Griseb.										0.1		0.1	
<i>Plantago tomentosa</i> Lam.										0.1	0.1	0.1	0.1

<i>Veronica serpyllifolia</i> L.																				0.1
<b>Poaceae C3</b>																				
<i>Chascolytrum lamarckianum</i> (Nees) Matthei																				0.1
<i>Chascolytrum poomorphum</i> (J. Presl) L. Essi, Longhi-Wagner & Souza-Chies																				0.1 0.1 0.1
<i>Chascolytrum rufum</i> J. Presl																				0.1 0.2
<i>Chascolytrum subaristatum</i> (Lam.) Desv.	0.1	0.1	0.2	0.1						0.1										0.1 0.2 0.2 0.2 0.1
<i>Chascolytrum uniolae</i> (Nees) L. Essi, Longhi-Wagner & Souza-Chies																				0.1 0.1 0.2
<i>Melica brasiliiana</i> Ard.																				0.1 0.1
<i>Nassella neesiana</i> (Trin. & Rupr.) Barkworth																				0.1
<i>Nassella nutans</i> (Hack.) Barkworth																				0.1 0.3 0.1 0.1 0.4
<i>Piptochaetium montevidense</i> (Spreng.) Parodi	0.3	0.5	0.2	0.3	0.6				0.1					0.1						0.3 0.6 0.4 0.3 0.3
<i>Piptochaetium ruprechtianum</i> E.Desv.																				0.1
<i>Piptochaetium stipoides</i> (Trin. & Rupr.) Hack. & Arechav.																				0.1 0.3 0.2 0.1 0.1
<i>Steinchisma hians</i> (Elliott) Nash	0.4	0.4	0.5	0.2	0.1	0.2	0.1	0.3	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2 0.1
<b>Poaceae C4</b>																				
<i>Andropogon bicornis</i> L.																				0.1 0.2
<i>Andropogon lateralis</i> Nees	0.2	1.1	1.6	1.1	2.0	0.2	0.7	0.8	1.1	1.6		1.0	0.5	0.5	2.7		0.4	0.4	0.9	1.4
<i>Andropogon selloanus</i> (Hack.) Hack.																				0.1 0.3 0.2
<i>Andropogon ternatus</i> (Spreng.) Nees																				0.1 0.2 0.1
<i>Aristida filifolia</i> (Arechav.) Herter																				4.0 1.9 1.0 0.1 1.9 2.4 0.2
<i>Aristida jubata</i> (Arechav.) Herter																				2.1 4.0 1.5 1.0 0.2 2.5 0.6
<i>Aristida laevis</i> (Nees) Kunth	0.5	1.6	1.3	0.6																0.5 1.4 1.7 0.1 2.0 0.1 0.9 1.0 1.5 0.3
<i>Aristida venustula</i> Arechav.																				2.0 1.0 0.2 0.5 0.1

<i>Axonopus affinis</i> Chase	0.7	0.5	0.5	1.0	0.1	0.4	0.2	0.4	0.4		0.2	0.3	0.5	0.6	0.1	0.2	0.2	0.3	0.7	0.1	
<i>Axonopus fissifolius</i> (Raddi) Kuhlm.															0.1						
<i>Dichantherium sabulorum</i> (Lam.) Gould & C.A.Clark		0.2	0.2	0.2	0.1		0.2	0.1	0.3	0.1				0.2	0.1		0.3	0.2	0.3	0.1	
<i>Digitaria violascens</i> Link					0.1					0.1											
<i>Elionurus candidus</i> (Trin.) Hack.																		0.1	0.7		
<i>Eragrostis airoides</i> Nees		0.2		0.1	0.1		0.1		0.1				0.1								
<i>Eragrostis bahiensis</i> Schrad. ex Schult.		0.1																0.1	0.2		
<i>Eragrostis lugens</i> Nees		0.4		0.2			0.1	0.1	0.1	0.1			0.1								
<i>Eragrostis neesii</i> Trin.	0.2	0.1	0.2			0.1		0.2				0.1					0.3	0.1	0.2		
<i>Eragrostis polytricha</i> Nees				0.4																	
<i>Microchloa indica</i> (L.f.) P. Beauv.	0.1																				
<i>Mnesithea selloana</i> (Hack.) de Koning & Sosef	0.4	0.2	0.1	0.1	0.1	0.3	0.6	0.3	0.1	0.1	0.1						0.3	0.2	0.2	0.2	0.1
<i>Panicum beergi</i> Arechav.					0.1																
<i>Panicum prionitis</i> Nees					0.6																
<i>Paspalum leptum</i> Schult.	0.1		0.2	0.1				0.2												0.2	
<i>Paspalum notatum</i> Flügge	2.9	1.3	2.1	1.0		2.4	0.4	2.0								1.9	1.0	1.0			
<i>Paspalum paniculatum</i> L.					8.3					9.0					9.0					9.0	
<i>Paspalum pauciciliatum</i> (Parodi) Herter	1.3	0.1	0.1													0.6				0.1	
<i>Paspalum paucifolium</i> Swallen	1.1			0.2	0.1	1.2	0.2		0.4	0.1						0.4					
<i>Paspalum plicatulum</i> Michx.	0.1	0.4	0.4	0.5	0.2		0.1		0.2	0.1		0.1		0.1	0.1	0.4	0.2	0.2	0.2	0.3	
<i>Paspalum pumilum</i> Nees		1.4	1.0	0.3		0.2	1.6	0.6	2.0				0.1			2.0					
<i>Saccharum angustifolium</i> (Nees) Trin.					6.0					6.0					4.5					6.0	
<i>Sacciolepis vilvoides</i> Chase																				0.1	0.2

<i>Schizachyrium condensatum</i> (Kunth) Nees	0.1	1.4	0.2	0.2	1.0	0.2	1.4	0.2										
<i>Schizachyrium spicatum</i> (Spreng.) Herter			0.1					0.2										
<i>Schizachyrium tenerum</i> Nees			0.1		0.7			0.1										
<i>Setaria parviflora</i> (Poir.) M. Kerguelen	0.2	0.2	0.3	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1	1.1	0.1	0.2	0.1
<i>Setaria vaginata</i> Spreng.	0.5	0.1	0.2	0.1	0.1	0.2	0.1	0.5	0.1	0.1	0.1	0.1	0.1	0.1	0.8		0.3	
<i>Sorghastrum setosum</i> (Griseb.) Hitchc.			1.0															1.0
<i>Sporobolus indicus</i> (L.) R.Br.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2										0.1
<b>Polygalaceae</b>																		
<i>Polygala aphylla</i> A.W.Benn.	0.1	0.1																0.1
<i>Polygala australis</i> A.W. Benn.										0.1	0.1	0.1		0.1	0.1	0.1		
<i>Polygala linoides</i> Poir.	0.1									0.1	0.1							
<i>Polygala pulchella</i> A.St.-Hil. & Moq.					0.1													
<b>Primulaceae</b>																		
<i>Lysimachia minima</i> (L.) U. Manns & Anderb.										0.1	0.1			0.1				
<b>Rubiaceae</b>																		
<i>Borreria brachystemonoides</i> Cham. & Schltdl.								0.1										
<i>Borreria dasycephala</i> (Cham. & Schltdl.) Bacigalupo & E.L.Cabral		0.1		0.1										0.2	0.1			0.1
<i>Galianthe fastigiata</i> Griseb.				0.2					0.1					0.1				0.2
<i>Galium humile</i> Cham. & Schltdl.				0.1					0.1					0.1		0.1		0.1
<i>Galium richardianum</i> (Gillies ex Hook. & Arn.) Endl. ex Walp.								0.1	0.1					0.1				0.1
<i>Richardia grandiflora</i> (Cham. & Schltdl.) Steud.			0.2	0.1	0.3				1.5	0.2				0.1				0.2
<i>Richardia humistrata</i> (Cham. & Schltdl.) Steud.	0.2	0.1	0.1	0.1	0.1	0.2	0.1		0.1	0.2				0.1	0.1			0.2
<i>Richardia stellaris</i> (Cham. & Schltdl.) Steud.	0.1	0.2	0.2	0.2		0.1		0.1							0.1			

<i>Spermacoce eryngioides</i> (Cham. & Schltld.) Kuntze	0.1	0.2					0.2	0.2			0.1		0.1	0.2	0.1			
<i>Spermacoce verticillata</i> L.	0.1	0.1	0.1	0.2	0.1		0.2	0.2	0.1	0.2	0.1		0.2	0.1	0.1	0.1	0.3	
<b>Turneraceae</b>																		
<i>Piriqueta suborbicularis</i> (A.St.-Hil. & Naudin) Arbo																0.1		
<b>Verbenaceae</b>																		
<i>Glandularia marrubioides</i> (Cham.) Tronc.													0.1		0.1	0.1	0.1	0.1
<i>Glandularia peruviana</i> (L.) Small																	0.1	
<i>Verbena ephedroides</i> Cham.						0.1			0.1	0.1			0.1				0.1	0.1

## CONSIDERAÇÕES FINAIS

Nosso estudo, compilados nos três artigos apresentados, é inovador em termos do conhecimento gerado sobre a dispersão de sementes e processos de floração e frutificação nos campos do Pampa brasileiro. Nós realizamos experimentos de coleta sementes por endozoocoria e da chuva sementes através de armadilhas com substrato estéril que geraram dados inéditos para os campos subtropicais da América do Sul. Também avançamos no conhecimento da fenologia e produção de sementes de ecossistemas campestre, propondo métodos para estimativa quantitativa da floração e frutificação ao longo de um ano e trabalhando numa perspectiva de restauração ecológicas. Os métodos de campo utilizados, em parte nunca aplicados no sistema de estudo, foram eficientes e forneceram dados que permitiram a análise de padrões e características acerca dos processos fenológicos e de dispersão para a vegetação submetida a diferentes intensidades de pastejo e exclusão do pastejo.

Em comum aos três estudos apresentados nos capítulos, nós observamos que a floração, a frutificação e a dispersão de sementes ocorrem, em nosso sistema do estudo, ao longo do ano inteiro, embora com clara variação da composição de espécies, em termos taxonômicos e funcionais, em resposta à sazonalidade do clima. O inverno apresentou menor número de plântulas nas amostras de esterco e chuva de sementes, bem como menor número de estruturas reprodutivas em relação à floração e frutificação. Também observamos que a composição de espécies variou entre às diferentes intensidades de pastejo e exclusão do pastejo. Inclusive identificamos que alguns grupos funcionais avançaram ou atrasaram a floração e frutificação conforme a variação entre as intensidades de pastejo.

Nossos resultados indicam que o gado pode ajudar a superar a limitação de sementes por endozoocoria em projetos de restauração. Esta contribuição do gado na dispersão de sementes não se limita a uma estação, mas ocorre ao longo do ano, com uma contribuição claramente menor apenas no inverno. Além da endozoocoria, o papel do gado se soma a outros

efeitos, como o fornecimento de matéria orgânica, o controle da biomassa vegetal por meio do consumo e pisoteio e abertura de espaço na vegetação. Níveis de pastejo moderado e baixo (8% e 12% FA) aparecem como tratamentos com maior contribuição de sementes, e a dispersão foi maximizada na primavera e verão, devido tanto à riqueza quanto à abundância de espécies e formas de vida na vegetação. A maioria das plantas que emergiram nas amostras de esterco foram as gramíneas e ciperáceas, o que é importante em termos de restauração, pois são os grupos funcionais que principalmente formam a matriz vegetal das comunidades campestres.

Também concluímos, com base dos dados de fenologia e da dispersão de sementes, que feno utilizado para fins de introdução de sementes – uma abordagem empregada com sucesso na Europa Central – deve ser coletado, nos Campos Sulinos, em vários momentos ao longo do ano, a fim de cobrir o amplo espectro de espécies das comunidades-alvo em pastagens subtropicais. Embora o momento exato da transferência de feno certamente varie entre locais específicos e também anos, os dados apresentados aqui podem ser considerados um primeiro passo para a definição de protocolos de transferência de feno, com transporte de sementes, de ambientes campestres na região. Novos estudos devem focar na quantidade e viabilidade das sementes, e também no volume adequado de feno a ser transferido em diferentes datas. As informações sobre o potencial e taxas de germinação também são importantes, assim como os testes sobre a melhor preparação dos locais receptores para aumentar o estabelecimento, como roçadas, por exemplo.

Os resultados do estudo sobre dispersão de sementes contribuem para a compreensão melhor da dinâmica da vegetação campestre, já que permitem avaliar melhor o potencial de recrutamento de indivíduos novos na vegetação. As armadilhas em potes com substrato estéril capturam as sementes efetivamente depositadas ou incorporadas ao solo, permitindo identificar quais espécies apresentam sementes viáveis e prontas para germinar nas condições locais. O que indica de forma realista quais espécies potencialmente atingem o solo e podem ser

incorporadas ao banco de sementes do solo, inclusive podendo servir como informação complementar para coleta de feno. Apesar das dúvidas em aberto sobre os melhores métodos para se estudar a chuva de sementes, nossos resultados mostram que um grande número de sementes é disperso e potencialmente incorporado ao banco de sementes do solo. Uma compreensão mais detalhada desses processos, possivelmente enfocando também as espécies-alvo, tem o potencial de melhorar o manejo do campo nativo, seja para uma perspectiva de conservação e restauração ou produção.

O estudo da fenologia das espécies campestres, da produção de sementes e de processos de dispersão e de recrutamento exigem um grande esforço amostral, em campo ou em casa de vegetação em laboratório. No entanto, este tipo de conhecimento é fundamental para avançar na restauração de áreas campestre no sul do Brasil. Além de estudos em nível da comunidade, sugerimos avaliar especificamente espécies-alvo da restauração, por exemplo de gramíneas que poderiam compor uma matriz inicial, ou de espécies com interesse para a conservação. Em projetos futuros, será importante ir além de estudos em parcelas pequenas, mas aplicar o conhecimento gerado no contexto real da restauração ecológica.



## ANEXOS

### Outras atividades desenvolvidas durante o doutorado

- 59th Annual Symposium of the International Association for Vegetation Science (IAVS). 12 a 17 de junho de 2016. Pirenópolis, Brasil.

Título: ‘The soil seed bank on different grazing intensities: restoration planning study’ (poster)

- 2º Botany Days. 25 a 27 de outubro de 2016. Porto Alegre, Brasil.

Comissão organizadora.

- 60th Annual Symposium of the International Association for Vegetation Science (IAVS). 20 a 24 de junho de 2017. Palermo, Itália.

Título: ‘The role of endozoochory dispersal by cattle in the transport of seeds in natural grasslands’ (resultados parciais. poster)

- 69º CNBot – Congresso Nacional de Botânica. 08 a 13 de julho de 2018. Cuiabá, Brasil.

Título: ‘O papel da dispersão endozoocórica pelo gado no transporte de sementes em pastagens naturais’ (resultados finais. poster)

- Participação no concurso ‘Dance Your Ph.D. 2016’, da revista Science, com a dança ‘The Gipsy Seeds’, disponível em <https://youtu.be/NDQmMBNURrI> e composição dos versos apresentados durante o vídeo:

*The gipsy seeds: A chuva de sementes e os ciganos*

As sementes são como os ciganos

Ambos se dispersam mundo a fora

Conquistando novos lugares e espaços

As adaptações morfológicas das sementes para facilitar a dispersão

São como os cabelos, saia e adereços da cigana

Leves e belamente adornados

Já os movimentos de dança

São como o vento

Envolvendo e carregando as sementes ao seu ritmo

Gracioso como uma brisa ou intenso como uma tormenta...

A dispersão é fundamental para sobrevivência

Nas plantas, de novos indivíduos

E nos ciganos, das tradições

E ao seu modo, garantido a sua riqueza e diversidade...

- Coorientação nos projetos dos bolsistas de iniciação científica Lucas Pinheiro (2016 – 2017)  
e Thiago Rambo (2018/1), alunos da Faculdade de Agronomia – UFRGS.